

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

John Goudie Ed.D.

## I. Overview:

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

General goals: Link science concepts with practical laboratory activity  
Provide students with immunoassay procedures and science behind them.

Placement: This instructor is using the activity in a Microbiology course where several chapters are devoted to Immunology. This could also be part of a forensic science unit.

Technical skills. Make dipsticks for assay; follow a multi-layered activity that takes more than one lab period; interview students in the simulated food poisoning activity to determine which foods were contaminated.

Relevance Common link to many newspaper articles about food poisoning outbreaks; Activity links outbreaks with determination of the cause.

## II. Science background:

Students, in my classes, will have has many laboratory sessions on microbial techniques, Immunology of specific immunity and immunization and Immunologic disorders and tests. However a general background of antigen and antibody activity and immunoassays for them probably is sufficient to understand the activity and get practical experience in a food poisoning investigation.

The techniques and materials and equipment are listed in the student lab.

## III. Student Outcomes:

Students will gain laboratory and real-world experience from a multi-layered activity that ranges from interviewing students from the simulated food poisoning event to solving the mystery of what foods caused the food poisoning from a specific immunoassay.

## IV. Learning Objects:

1. Students will learn to interview patients and collect and tabulate pertinent data for analysis of the food poisoning event. Reports will be submitted.
2. Students will gain firsthand experience with antigen-antibody testing and the reasons for the procedures by means of a test.
3. Students will gain experience in relating immunoassays to clinical observations. Laboratory grades will be assigned for student work in the la.

## V. Time Requirements:

1. The lab investigation can be held in 2-70 minute lab blocks, but has stopping points for shorter lab periods.

## VI. Advance Preparations:

Equipment: 37° Incubator  
Nitrocellulose  
Double –face tape  
Acetate film (transparency)  
Micropipetors  
Small Tupperware containers or Petri dishes

### Solutions:

#### 1. PBS (Phosphate buffered saline)(Washing buffer)

10X PBS (0.1M PBS, pH 7.4):

Na<sub>2</sub>HPO<sub>4</sub> (anhydrous) ----- 10.9 g

NaH<sub>2</sub>PO<sub>4</sub> (anhydrous) ----- 3.2 g

NaCl ----- 90 g

Distilled water ----- 1000 ml

Mix to dissolve and adjust pH to 7.4

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

1. TMB peroxidaase substrate  
Promega, Corp

TMB Stabilized Substrate for Horseradish

Peroxidase 200ml W4121 \$89.00

2. 5% Not-fat dairy milk-local store

3. Tween in PBS

10X PBS-Tween 20 (0.1M PBS, 0.5% Tween 20, pH 7.4):

Na<sub>2</sub>HPO<sub>4</sub> (anhydrous) ----- 10.9 g

NaH<sub>2</sub>PO<sub>4</sub> (anhydrous) ----- 3.2 g

NaCl ----- 90 g

Distilled water ----- 1000 ml

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: BSA A2-153-10g \$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 important to understand any immunoassay.
- b. This is a low tech lab investigation

## **IX. What is expected from students:**

- a. Food Investigation Report
- b. Food Investigation tally sheet with determination of suspected foods
- c. Presentation of results confirming suspected foods containing antigens.
- d. Understand the procedure used

## **X. Anticipated results**

- a. Sufficient to determine potential sources of antigen in foods
- b. Correct determination of antigen present in simulated food samples



## **XI. Classroom discussion**

- a. Discussion of other immunoassays like Elisa Test, Rocket Immunoassays, Western Blots
- b. Discussion of available dip stick immunoassay test

## **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. Detection of presence of cow's milk in sheep's cheeses 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)2479-2483.
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

[http://www.co.polk.or.us/sites/default/files/community\\_development/Food%20Investigation%20Report%20Form.pdf](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](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](http://www.who.int/foodsafety/publications/foodborne_disease/Annex_6.pdf)

4. Polk County, Oregon Food Investigation Report  
[http://www.co.polk.or.us/PH\\_FoodReport](http://www.co.polk.or.us/PH_FoodReport)

## **Student Section**

### **I. Rationale**

This activity was designed to teach within an Immunology Unit with the goal of integrating realistic aspects of solving a food poisoning mystery using both epidemiology and immunology techniques. Knowledge of important aspects of disease surveillance including data collection by surveys and laboratory experience will be used to identify the causative agent.

### **II. Materials per group**

#### **Part A Food Poisoning Outbreak**

1. Food Poisoning Scenario similar to the following: Mr. and Mrs. Mott allowed their daughter, Julia, to have a party for her sixteenth birthday. The party was to begin at 6:00 pm in the back yard, as it was springtime. At this party, Mrs. Mott and her daughter prepared the following: potato salad, fruit salad, hot dogs and hamburgers, various sodas, coffee and tea, sausage and queso chip-dip, birthday cake, and ice cream. The party ended at 11:00 pm.

2. List of students to interview: obtained from your instructor.

2. Investigator worksheet

3. Master List worksheet

#### **Part B Dipstick Immunoassay**

1. Simulated food samples with primary antigen

2. 1X PBS ( Phosphate buffered saline)(Washing buffer)

3. Nitrocellulose sheets.

4. 5% Not-fat dairy milk

**6. Tween in PBS**

**7. Simulated food samples of selected foods from your instructor.**

### **III. Procedure**

#### **Part A Food Surveillance Procedure**

1. Interview the victims assigned to you and complete the Investigator work sheets\*.

2. Tally all the worksheets and transfer data to the Master List\*.

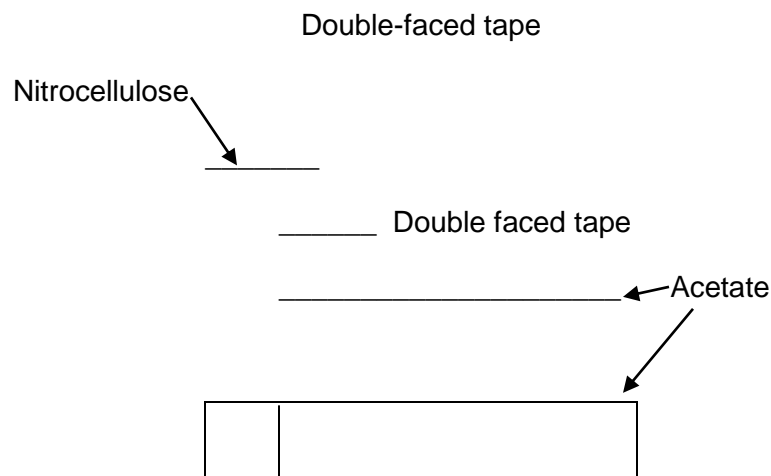
3. Confer with colleagues to determine source of contaminated food.

\* Worksheets attached.

### Part B. Dipstick Immunoassay ( See Teacher Notes in Appendix)

#### Preparation of Dipsticks

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.



**Fig. 1** Dipstick assembly: the nitrocellulose membrane is pasted to the plastic stripe with an adhesive tape.

#### Procedure Part 2 Methodology for testing serum.

1. Make nitrocellulose dip-strips attached to acetate ( See preparation of nitrocellulose strips).
2. Dip strips in 5% ethical alcohol to wet. Shake off excess alcohol. Dry strips for 5 minutes.
3. Add 1-5 ul of primary antigen a line (or a drop) near the reaction end of the nitrocellulose strip. Let dry in incubator until dry. The primary antigens in this lab are the food specimens assigned you and your partner.

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 blocking 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 % non 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).
11. Add enzyme TMB Peroxidase substrate. Careful not to overexpose or entire reaction end will stain. (TMB Peroxidase substrate is enzyme substrate applied and the resulting colored precipitate localizes the antigen as a colored band or spot on the membrane).
12. Water will stop the reaction in step 10. **Record + fir positive reactions for *Bacillus cereus* type B enterotoxin or - if not present in the food sample tested.** Record your finding on the Results Section

#### IV. Data Collection

##### Part B.

#### Immunoassay for Presence Suspected Antigen

<b>Food Sample #</b>	<b>Group #</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>
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Table #1 Record + of positive reaction for *Bacillus cereus* type B enterotoxin or negative for not present in the food sample tested.

#### V. Discussion Analysis

1. Is there a correlation between the foods suspected of being contaminated with the results of the immunoassay? Which foods correlate and which do not? Why
2. What variables might interfere with the dipstick assay techniques?
3. Now that you know the contaminated food and the agent? What do you do now?



## **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 carbohydrates, 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 37oC 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 @ 37oC or overnight at 4oC.
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.



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## Food

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

Available in [PDF \(313KB\)](#).<sup>1</sup>

Also available [en Español \(Spanish\)](#).<sup>2</sup>

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 each year. And each year these illnesses result in an estimated 128,000 hospitalizations and 3,000 deaths.

The chart below includes foodborne disease-causing organisms that frequently cause illness in the United States. As the chart shows, the threats are numerous and varied, with symptoms ranging from relatively mild discomfort to very serious, life-threatening illness. While the very young, the elderly, and persons with weakened immune systems are at greatest risk of serious consequences from most foodborne illnesses, some of the organisms shown below pose grave threats to *all* persons.

Organism	Common Name of Illness	Onset Time After Ingesting	Signs & Symptoms	Duration	Food Sources
<i>Bacillus cereus</i>	<i>B. cereus</i> food poisoning	10-16 hrs	Abdominal cramps, watery diarrhea, nausea	24-48 hours	Meats, stews, gravies, vanilla sauce
<i>Campylobacter jejuni</i>	Campylobacteriosis	2-5 days	Diarrhea, cramps, fever, and vomiting; diarrhea may be bloody	2-10 days	Raw and undercooked poultry, unpasteurized milk, contaminated water
<i>Clostridium botulinum</i>	Botulism	12-72 hours	Vomiting, diarrhea, blurred vision, double vision, difficulty in swallowing, muscle weakness. Can result in respiratory failure and death	Variable	Improperly canned foods, especially home-canned vegetables, fermented fish, baked potatoes in aluminum foil
<i>Clostridium perfringens</i>	Perfringens food poisoning	8–16 hours	Intense abdominal cramps, watery diarrhea	Usually 24 hours	Meats, poultry, gravy, dried or precooked foods, time and/or temperature-abused foods
<i>Cryptosporidium</i>	Intestinal cryptosporidiosis	2-10 days	Diarrhea (usually watery), stomach cramps, upset stomach, slight fever	May be remitting and relapsing over weeks to months	Uncooked food or food contaminated by an ill food handler after cooking, contaminated drinking water
<i>Cyclospora cayetanensis</i>	Cyclosporiasis	1-14 days, usually at least 1 week	Diarrhea (usually watery), loss of appetite, substantial loss of weight, stomach cramps, nausea, vomiting, fatigue	May be remitting and relapsing over weeks to months	Various types of fresh produce (imported berries, lettuce, basil)
<i>E. coli</i> ( <i>Escherichia coli</i> ) producing toxin	<i>E. coli</i> infection (common cause of “travelers’ diarrhea”)	1-3 days	Watery diarrhea, abdominal cramps, some vomiting	3-7 or more days	Water or food contaminated with human feces

<i>E. coli</i> O157:H7	Hemorrhagic colitis or <i>E. coli</i> 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 vegetables (e.g. sprouts), and contaminated water Raw produce, contaminated drinking water, uncooked foods
Hepatitis A	Hepatitis	28 days average (15-50 days)	Diarrhea, dark urine, jaundice, and flu-like symptoms, i.e., fever, headache, nausea, and abdominal pain	Variable, 2 weeks-3 months	and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters
<i>Listeria monocytogenes</i>	Listeriosis	9-48 hrs for gastro-intestinal symptoms, 2-6 weeks for invasive disease	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth. The elderly or immunocompromised patients may develop bacteremia or meningitis.	Variable	Unpasteurized milk, soft cheeses made with unpasteurized milk, ready-to-eat deli meats
Noroviruses	Variously called viral gastroenteritis, winter diarrhea, acute non-bacterial gastroenteritis, food poisoning, and food infection	12-48 hrs	Nausea, vomiting, abdominal cramping, diarrhea, fever, headache. Diarrhea is more prevalent in adults, vomiting more common in children.	12-60 hrs	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters
<i>Salmonella</i>	Salmonellosis	6-48 hours	Diarrhea, fever, abdominal cramps, vomiting	4-7 days	Eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated raw fruits and vegetables Raw produce, contaminated drinking water, uncooked foods
<i>Shigella</i>	Shigellosis or Bacillary dysentery	4-7 days	Abdominal cramps, fever, and diarrhea. Stools may contain blood and mucus.	24-48 hrs	and cooked foods that are not reheated after contact with an infected food handler
<i>Staphylococcus aureus</i>	Staphylococcal food poisoning	1-6 hours	Sudden onset of severe nausea and vomiting. Abdominal cramps. Diarrhea and fever may be present.	24-48 hours	Unrefrigerated or improperly refrigerated meats, potato and egg salads, cream pastries
<i>Vibrio parahaemolyticus</i>	<i>V. parahaemolyticus</i> infection	4-96 hours	Watery (occasionally bloody) diarrhea, abdominal cramps, nausea, vomiting, fever	2-5 days	Undercooked or raw seafood, such as shellfish
<i>Vibrio vulnificus</i>	<i>V. vulnificus</i> infection	1-7 days	Vomiting, diarrhea, abdominal pain, bloodborne infection. Fever, bleeding within the skin, ulcers requiring surgical removal. Can be fatal to persons with	2-8 days	Undercooked or raw seafood, such as shellfish (especially oysters)

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 (toll free), 10 AM to 4 PM ET, Monday through Friday.**

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**Links on this page:**

1. </downloads/Food/ResourcesForYou/Consumers/UCM187482.pdf>
2. </Food/ResourcesForYou/Consumers/ucm250640.htm>

## 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: \_\_\_\_\_)**

# FOOD INVESTIGATION REPORT

NAME \_\_\_\_\_

DATE \_\_\_\_\_

Anything you drank. (time for each)

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

Do you know anyone [else] that was ill? (name, address, phone number for each)

Let me read you a list of symptoms. For each one, give me a "yes" or "no." Did you have any...

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B	<input type="checkbox"/>	<input type="checkbox"/>	Did you notice any blood in stools?																																																		

X    Any other noteworthy symptoms? *If yes, specify* \_\_\_\_\_

**On what day did you first have any vomiting or diarrhea? At what time?**

*(Record date and time below. Be sure to clarify times such as "midnight" or early morning hours—make sure you indicated the correct day. Midnight exactly is the end of the day. Prompt as needed to get a precise time estimate, e.g. "What is your best guess of the time?"*

**DAY**     day1         day2         day3         day4         day5         \_\_\_\_\_

**TIME** *(circle closest hour—make sure you indicate AM or PM correctly)*

<b>AM</b>	0	1	2	3	4	5	6	7	8	9	10	11	12
	>midnight												noon
<b>PM</b>	12	1	2	3	4	5	6	7	8	9	10	11	12
	>noon	13	14	15	16	17	18	19	20	21	22	23	midnight

*If no vomiting or diarrhea, when did you first become ill? (Write in)*

**How long did you feel sick?** \_\_\_\_\_ (specify...  days     hours )     still sick

**Did you...** *(check all that apply; provide details [names, dates, phone numbers, etc.] at right.)*

Y	N	??	
W	<input type="checkbox"/>	<input type="checkbox"/>	miss work or school? <i>If yes, how many days</i> _____
S	<input type="checkbox"/>	<input type="checkbox"/>	give a stool specimen? <i>If yes, to whom</i> _____
P	<input type="checkbox"/>	<input type="checkbox"/>	see a physician/HCP <i>If yes, who?</i>
E	<input type="checkbox"/>	<input type="checkbox"/>	visit an ER <i>If yes, specify</i>
H	<input type="checkbox"/>	<input type="checkbox"/>	get admitted overnight to hospital <i>If yes, specify</i>
D	<input type="checkbox"/>	<input type="checkbox"/>	die <i>If yes, express condolences.</i>

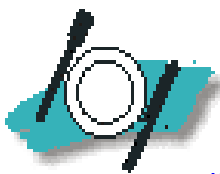


Department of  
Health

**FOOD SAFETY BRANCH**

# Standard Operating Procedure for the Investigation of Food Poisoning Outbreaks

*Rural and Regional Edition*



Visit Food Safety online

<http://www.public.health.wa.gov.au/>

## 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:

- Incidents exceeding 2 separate but related cases **AND**
- Infected persons living in more than one local government area **OR**
- Wide public health risk (eg a food product having a statewide distribution) **OR**
- Nature / species of food poisoning organism involved (eg s.typhoid)

The [Outbreak decision tree \(Chart 2\)](#) is a guide to assess such a complaint.

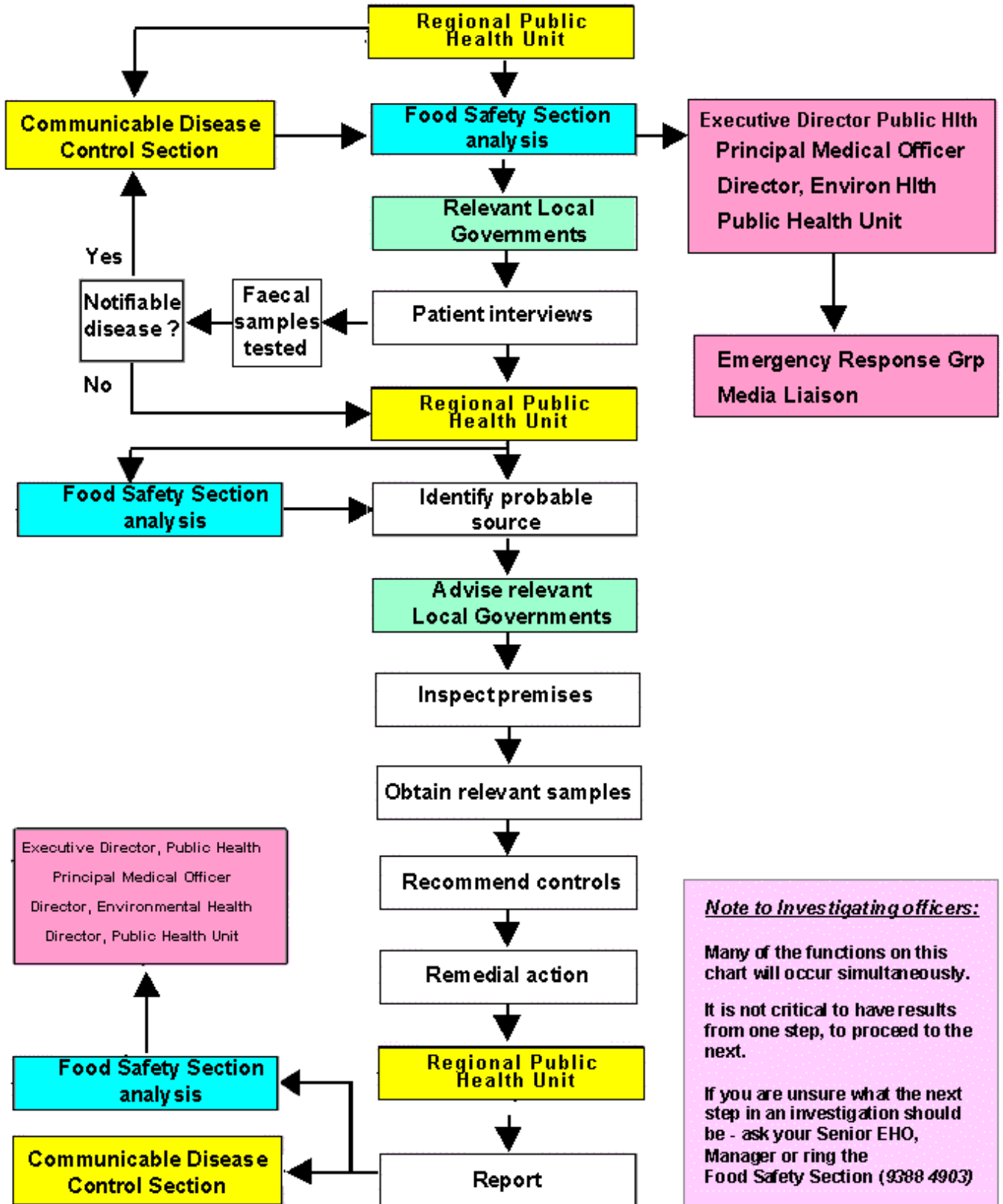
For new (associated) enteric diseases not exceeding 2 separate but related cases, it is appropriate for the local government EHO to carry out the investigation and follow up. However a Public Health Unit should be advised of the outcome, which allows staff to remain informed and notification details to be updated in the WANNID system. It also allows a reported illness to be upgraded to outbreak if further complaints are received. The Food Safety Section is available for any advice or support that may be required.



Chart 1

## *Outbreak Flow Chart*

Regional areas  
*(Chart 1)*



***Note to Investigating officers:***

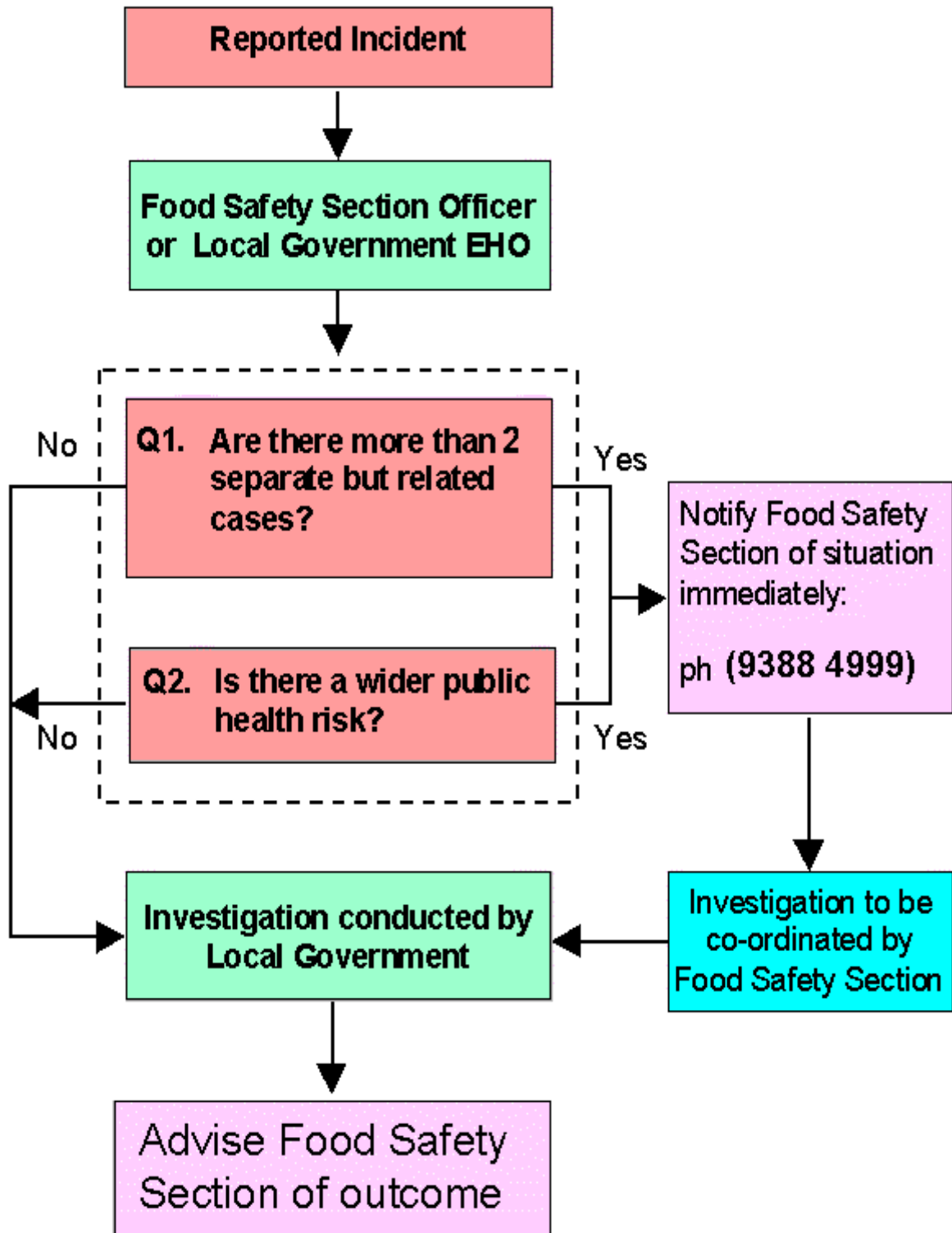
Many of the functions on this chart will occur simultaneously.

It is not critical to have results from one step, to proceed to the next.

If you are unsure what the next step in an investigation should be - ask your Senior EHO, Manager or ring the Food Safety Section (9388 4903)

Chart 2

## *Outbreak Decision Tree (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.

<b>PUBLIC HEALTH UNITS</b>
<b>Coastal &amp; Wheatbealt (08) 9622 0120</b>
<b>Gascoyne (08) 9941 0560</b>
<b>Goldfields (08) 9021 2622</b>
<b>Great Southern (08) 9841 8244</b>
<b>Kimberley (08) 9191 1144</b>
<b>Midwest (08) 9964 4299</b>
<b>Pilbara (08) 9140 2377</b>
<b>Southwest (08) 9792 2500</b>
<b>The Food Safety reception number is (08) 9388 4903 or fax Food Safety on (08) 9382 8119</b>

### 3. Referral to Local Government

During an investigation of an outbreak it is critical that clear notes be kept detailing all steps taken during the investigation. These notes should include:

- Completed questionnaires,
- Assessment of all potential risk factors,
- Details of any initial control measures implemented,
- Any other action taken including a summary of findings

Local government should keep their own records for future reference and in the event that council determines to prosecute the owner of the premises, or the EHO is called as an expert witness in cases of civil litigation in support of damages claims for persons who became ill.

Cases for follow up will be referred to the relevant local government, in which the case resides. This will be coordinated by the Public Health Unit in regional areas and the Food Safety Section in Metropolitan areas. An investigator may need to refer the inspection forms of food premises, special care facilities, child care centres etc. to other local governments when a need for follow up is indicated, as details emerge.

In these circumstances, the investigation report should reflect who information was referred to and at what point in the investigation.

If the outbreak is in a regional area, the relevant Public Health Unit Coordinator **must** also be advised of the situation.

### 4. Patient interviews

Upon receiving information relating to what appears to be an outbreak, an investigating officer should attempt to gather a complainant's details and if known, any other contact details of affected persons.

- Full name
- Date of birth (DOB),
- Address,
- Phone number,
- Foods consumed,
- The time of onset,
- Symptoms experienced,
- GP seen / faecal sample submitted? (if applicable)

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 completed**, so any other possible sources can be identified. Location, day, date, time and company involved (if known) should be established. Copies of the EDIR form are available from Food Safety, or (see [attachment 1](#)).

It is important when conducting an investigation into an alleged outbreak to collect a detailed dietary history of foods consumed at an event. It is likely that many other people have eaten similar dishes. One food type may be implicated by examining dietary histories, or the possibility of cross contamination.

**NOTE:** Filling in all fields on an EDIR form, (even if the answers seem unlikely sources), may assist other officers in finding a cause. Some atypical foods have been associated with major food poisoning outbreaks eg orange juice & peanut butter.

It can be valuable to obtain a menu from the event or premises to assist with the investigation, often people may not remember fully what they consumed. With a menu that can be shown or read to them, an affected person's recall may be enhanced and allow a more accurate analysis of food consumed vs illness.

Obtaining a copy of the restaurant booking list is also of great value for contacting possible cases and EHOs have the power to request it, but discretion **MUST** be exercised in the use of this information.

Interview as many affected people as possible – a detailed dietary history of multiple cases allows a more clear direction to be adopted when following up the complaint with a food business. Also ask people if they

noticed any poor food handling practices at the event – sometimes this information can prove invaluable.

Local government EHOs should quickly convey any affected persons details not interviewed by them and not living in their area of responsibility, to the Public Health Unit for regional cases, or the Food Safety Section for metropolitan cases.

## **6. Faecal specimens needed to confirm causative agent (s)**

The Food Safety Section has a policy whereby a faecal specimen is collected from all affected people where possible. Officers from Food Safety or local government EHOs may drop off and collect 'yellow cap' specimen jars for analysis and confirmation of illness. If affected persons have not already submitted a sample for analysis through their GP, DOH will offer this service. Currently the PathCentre is the approved analyst for faecal specimens.

Prior to collection of these samples, the Medical Officer in Disease Control, or the Chief Medical Officer at the Public Health Unit must consent to their provider number being used for the sample analysis. Investigating officers must ensure that consent has been given for this prior to issuing jars and collecting samples.

Any food handlers from the event who are experiencing similar symptoms, or have had an enteric illness recently should be asked for a faecal sample at the same time as affected persons who attended the event. Food Safety has a target of as many faecal specimens as is possible to be collected ASAP per outbreak, either through people submitting through their GP or by special arrangement with DOH

A blank copy of the new PathCentre faecal submission form is available in attachment 2 (an example of a correctly filled in form is also provided).

**This form must be filled out COMPLETELY – including the patient's name, age, address details, phone number, medicare number & signature etc.**

**DO NOT** fill out the doctors details, this must be done by a GP, one of Disease Control's public health Doctors, or a Doctor at a Public Health Unit - PathCentre automatically refers unsigned forms to Department of Health.

## **7. Obtain relevant food samples**

If any food remains from a function or event where an alleged food poisoning outbreak has taken place, then it is important to arrange sampling of that remaining food for analysis. A sample of food from the event may provide a strong link between food consumed and disease.

Importantly products with the same batch number, or production date should be looked for during a follow up inspection and considered relevant for sampling. Food handlers may be able to reveal when a product or batch was made.

Question staff and management as to whether the food served was pre-made and reheated or prepared freshly for the function. This information may reveal poor food handling practices in preparation, storage, temperature controls and transport.

Seizure of remaining foods may also be appropriate, however it should be discussed with the officer's PEHO or the Food Safety Section beforehand.

## **8. E-mail briefing**

At this point in the investigation important facts regarding the size and probable nature of the outbreak have emerged. Considerable information is known and evidence of the symptoms and circumstances of the outbreak have been verified. It is important that this information is provided to the Department of Health and so that it may be distributed to the relevant officers such as the Executive Director of Public Health, Director of Environmental Health, Director of Communicable Disease Control and Manager of Public Affairs.

An email briefing summarising all activities undertaken by the investigating officers to date must be produced and send to the Coordinator of the outbreak investigation in the Food Safety Branch, who will revise the information and distribute the summary to all key officers.

Emails are to be in a simple standard format with the objective that the information may be easily compiled to prepare the final investigation report (see 11. Generate report on investigation.).

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.)

## **9. Identification of causative pathogen**

Laboratory confirmation of causative pathogen is a vital step in the investigation of a food borne outbreak. By sampling any remaining foods and obtaining faecal specimens from affected persons, an investigating officer has a greater chance of determining the causative organism.

If a causative organism cannot be linked from food or faecal samples, this does not mean that a food poisoning outbreak has not occurred – only that the samples tested were not able to reveal a pathogen. More detailed or specific testing may be required.

Food & faecal samples should be submitted to a laboratory, for analysis, with a request that a result is made available ASAP. Requests for a PCR viral test on faecal samples may also be appropriate – however this should be discussed with Food Safety first.

Once an organism or pathogen has been identified from food or faeces samples, the investigation must focus on what food handling practices may have caused the outbreak.

## **10. Recommend and Implement control measures**

Once the causative organism has been identified, it is imperative that any further infection is prevented. These are functions that an EHO may perform to prevent any other cases:



- Seizure of remaining foodstuffs / overseeing destruction of remaining foodstuffs
- Directing cleaning and sanitising of a food premises
- Implementing changes in food handling practices in a food business
- Obtaining EDPH consent to exclude a food handler from work
- Seeking EDPH consent to close a food business
- Ensuring water supply is safe to drink (if water borne)
- Providing general hygiene advice to affected persons
- Suggesting the implementation of a food safety plan within the food business, to control risks

**REMEMBER:** Any higher level control actions such as closure of a business and exclusion of a food handler from work, must be approved by the EDPH.

## **11. Generate report on investigation**

At the conclusion of an investigation into a food poisoning outbreak, a report must be written by investigating officers incorporating information gleaned from the 10 data objectives.

The final report should be concise, approximately 5 - 8 pages, and contain the following information:

- Title: Geographical and premises location of outbreak
- Investigating Officer name, phone number and email address.
- Background information
- Investigation method and results
- Discussion on the outcome of the investigation and to what degree the cause was identified, the impact on the people affected, how the situation might have been avoided, what was undertaken immediately to prevent any further cases of illness and what needs to be implemented to prevent the situation occurring in the future.
- Conclusions
- Recommendations

Reports and notes made during any investigation should be filed correctly together. For Food Safety Section officers, all notes, results and reports must be filed on the current Food Poisoning file.

**The final report, or interim report if investigations are uncompleted, must be presented within 14 days from the date of being notification of the outbreak.**

### **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 sickness has caused. Information may be sought through approved channels, by lawyers acting on the affected person's behalf or others, 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 release 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 Food Safety Branch when an alleged food poisoning incident is reported.

- **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? (What premises – country or metro?)
  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)
- Refer to [Chart 2](#) in outbreak handout to determine if this is an outbreak

### **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 (**PHS – Food safety** in outlook list) & Medical Epidemiologist listing the above details.
3. If further action is deemed necessary, Food Safety Section Officer to notify LG – speak to the Principal Environmental Health Officer and area Environmental Health Officer.
4. Arrange inspection of food premises by the area EHO ASAP.
5. If it is apparent that there is an ongoing risk of food poisoning (e.g. ongoing temperature abuse of hazardous foods) determine if immediate corrective action can be taken to permit the premises to continue operating.
6. Ask EHO to obtain relevant food samples (if available).
7. **Seek** to exclude any food handler, childcare worker or children who have active symptoms of food poisoning in accordance with [exclusion guidelines](#).
8. Provide information and educational resources for managers and staff .
9. If faecal samples have not already been collected by a person's GP, Food Safety Section may request local EHOs to do so. The Medical Epidemiologist must be consulted prior to doing this, as their provider number will be required.
10. Briefing to be organised with LG EHOs and Food Safety Section officers.

## **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.

- **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)
- Refer to [Chart 2](#) in outbreak handout to determine if this is an outbreak

### **Administrative action – Local Government EHOs**

1. Discuss situation with PEHO / SEHO.
2. Obtain last inspection report of food premises (if available) and determine if there were any major defects noted or work orders issued and when.
3. Phone Regional Public Health Unit and advise them of situation and request support if appropriate.
4. Phone Food Safety Section – 9388 4903.
5. Arrange inspection of food premises by area EHO, ASAP.
6. Obtain relevant food samples from premises – if applicable. Phone Food Safety Branch and discuss what samples are to be tested.
7. Consult with Regional Public Health Unit regarding the exclusion of any food handler, child care worker or children who have active symptoms of food poisoning in accordance with [exclusion guidelines](#)
8. Provide information and educational resources for managers and staff.
9. If faecal samples have not already been collected by a person's GP, Food Safety Section may request you to do so – the Medical Epidemiologist, must be consulted prior to doing this, as their provider number will be required.
10. Briefing to be organised with Local Government, EHOs and Regional Public Health Officers.
11. Prepare a report on incident and outcome and advise Food Safety Branch.

### 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.

- **Date of report**

- **Names and affiliations of the main authors and investigators**

#### Abstract

The abstract should be written after the report has been completed. It should stand alone and contain the most relevant data and conclusions. All data mentioned in the abstract must also appear in the main section of the report. Sentences from the Discussion section can be used verbatim in the abstract.

#### Report

- **Introduction**

Statement of the problem and its public health importance.

Details and time frame regarding initial source of information.

Reasons for investigating event.

Type of investigations conducted and agencies involved.

- **Background**

Generally available information to help the reader interpret epidemiology and data presented in the report (e.g. population size, socioeconomic status of community, ethnicity, etc.).

If outbreak occurred in a food premises, description of premises (e.g. size of restaurant, usual practices and operations, etc.).

Description of the problem.

Sequence of events leading to the study or investigation.

Brief statement of the working hypothesis.

- **Objectives**

Specify targets to be achieved by the investigations.

Keep objectives concise and follow a logical, sequential pattern.

The objectives may include hypotheses, if any, to be tested.

- **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
- name of laboratory carrying out tests
- laboratory techniques employed and methods of data analysis.

Food and food testing:

- description of inspection process
- methods of food and environmental sampling
- name of laboratory carrying out tests
- laboratory techniques employed and methods of data analysis.

- **Results**

Present all pertinent results from clinical, laboratory, epidemiological and environmental findings.

Present results in same order as described in the methods section.

Do not interpret or discuss the data in this section.

Epidemiology:

- number of cases, overall attack rate
- clinical details of illness (symptoms, duration, hospitalization, outcome, etc.)
- descriptive epidemiology by time (epidemic curve), place and person (age, sex, race, specific characteristics) expressed as rates
- risk factor exposures
- further data analysis and data presentation depending on specific studies undertaken (e.g. cohort or case-control study).

Laboratory (microbiology, chemical, toxicological):

- number of specimens collected
- findings by type of laboratory analysis.

Food investigation and food testing:

- findings of food inspections
- results of laboratory tests performed on food and environmental samples.

- **Discussion**

The discussion is the most important part of the report and should cover:

- summary of the major findings
- likely accuracy of the results



- 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.



**12. Place where food was contaminated:**  
 Place: code  Country: code

---

**13. Place and date where food was acquired and eaten:**  
 Date: \_\_\_ / \_\_\_ / \_\_\_\_\_ Place: code   
           day month year  
 During transit:  
 Means of transit: code  from: code  to: code

---

**14. Factors contributing to incident:**  
 (a) Code  (b) Code   
 Other  
 \_\_\_\_\_  
 \_\_\_\_\_  
*Note: In case more than one factor contributed, list all that are applicable but code only the two major factors.*

---

**15. Results of lab. tests:**  
 Testing laboratory: \_\_\_\_\_

Specimens/samples	No. tested	Positive	Details/comments
Ill people*	_____	_____	_____
Well people*	_____	_____	_____
Food-handlers	_____	_____	_____
Suspect food	_____	_____	_____
Other foods	_____	_____	_____
Environment	_____	_____	_____

\* Clinical samples.

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

OUTBREAK NO. 97\.....

Name: \_\_\_\_\_ Address: \_\_\_\_\_  
Position: \_\_\_\_\_  
Telephone: \_\_\_\_\_ LA: \_\_\_\_\_ DHA: \_\_\_\_\_  
Date: \_\_\_\_\_

**1. MODE OF TRANSMISSION** (tick one only)

- Mainly person to person  Mainly foodborne   
Equal or unknown proportion of foodborne and person to person   
Other  Specify water, animal contact, etc. \_\_\_\_\_  
Unknown

**2. PLACE WHERE OUTBREAK OCCURRED, or if foodborne where food was prepared or served.** Tick one only. If foodborne "PREPARED" takes precedence over "SERVED", e.g. if food was prepared in a shop but served in a house, tick "Shop/retailer", if food was prepared at a house and served elsewhere, tick "Private house".

- (a) Private house   
(b) House/guest house/residential pub  Specify \_\_\_\_\_  
(c) Restaurant/café  Specify ethnicity \_\_\_\_\_  
(d) Pub/bar   
(e) Mobile retailer  Specify market trader, chip van, etc. \_\_\_\_\_  
(f) Armed services camp  Specify army, navy, etc. \_\_\_\_\_  
(g) Canteen  Specify work, college \_\_\_\_\_  
(h) Shop/retailer  Specify baker, butcher, etc. \_\_\_\_\_  
(i) Hospital  Specify general, geriatric, EMI \_\_\_\_\_  
(j) Residential institution  Specify nursing/residential home \_\_\_\_\_  
(k) School  Specify nursery, junior, etc. \_\_\_\_\_  
(l) Other  Specify \_\_\_\_\_

**3. NAME AND ADDRESS OF PLACE** \_\_\_\_\_  
\_\_\_\_\_ Postcode (if known) \_\_\_\_\_

**4. WAS THE OUTBREAK AT A FUNCTION?** Yes  No  Date of function \_\_\_/\_\_\_/\_\_\_

**5. WAS PATHOGEN/TOXIN IDENTIFIED?** Yes  No

If YES give: Organism/toxin \_\_\_\_\_ Serotype \_\_\_\_\_ Phage type \_\_\_\_\_  
If NO: Specify organism suspected \_\_\_\_\_

**6. LABORATORY where tests performed:** State first and reference labs, even if microbiology was negative

\_\_\_\_\_  \_\_\_\_\_  
First lab Reference lab

7. **TOTAL NUMBER AFFECTED** (diarrhoea and/or vomiting +/- any other symptom) \_\_\_\_\_

**TOTAL NUMBER AT RISK** \_\_\_\_\_

Number admitted to hospital \_\_\_\_\_

Number known to have died \_\_\_\_\_

**8. LABORATORY RESULTS**

NUMBER OF PEOPLE	AFFECTED PEOPLE		WELL PEOPLE	
	TESTED	POSITIVE	TESTED	POSITIVE
8a. <i>HOSPITAL OR RESIDENTIAL OUTBREAKS ONLY categories (i) and (j) in question 2</i>				
Residential/patients				
Staff				
Total				
8b. <i>ALL OTHER OUTBREAKS</i>				
Non-food-handlers				
Food handlers				
Total				

9. **DATE OF ONSET:** First known \_\_\_/\_\_\_/\_\_\_\_ Last known \_\_\_/\_\_\_/\_\_\_\_

10. **SUSPECT FOOD VEHICLE ASSOCIATED WITH ILLNESS:** only list specific vehicle for which there is microbiological, statistical or other convincing association with illness.


VEHICLE	EVIDENCE (tick)		
	Microbiological	Statistical association	

**11. FAULTS THOUGHT TO HAVE CONTRIBUTED TO OUTBREAK:**

- Infected food-handler  Give details \_\_\_\_\_
- Inadequate heat treatment  Give details \_\_\_\_\_
- Cross contamination  Give details \_\_\_\_\_
- Storage too long/too warm  Give details \_\_\_\_\_
- Other  Give details \_\_\_\_\_

Environmental Health Department's inspection rating of premises (if available) (A-F): \_\_\_\_\_

Foodborne disease outbreak report form from Centers for Disease Control and Prevention, USA

 <p>Electronic Foodborne Outbreak Reporting System</p>	<h3>Investigation of a foodborne outbreak</h3>	CDC Use Only _____
	<p>This form is used to report foodborne disease outbreak investigations to CDC. It is also used to report <i>Salmonella enteritidis</i> and <i>E. coli</i> O157:H7 outbreak investigations involving any mode of transmission. A foodborne outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food in the United States. This form has 6 parts. Part 1 asks for the minimum or basic information needed and must be completed for the investigation to be counted in the CDC annual summary. Part 2 asks for additional information for any foodborne outbreak, while Parts 3–6 ask for information concerning specific vehicles or etiologies. Please complete as much of all parts as possible.</p>	State Use Only _____

**Part 1: Basic information**

<p><b>1. Report type</b></p> <p>A. <input type="checkbox"/> Please check if this is a final report</p> <p>B. <input type="checkbox"/> Please check if data does not support a FOODBORNE outbreak</p>	<p><b>3. Dates</b></p> <p>Please enter as many dates as possible</p> <p>Date first case became ill                  ___/___/___                  Month Day Year</p> <p>Date last case became ill                  ___/___/___                  Month Day Year</p> <p>Date first known exposure                  ___/___/___                  Month Day Year</p> <p>Date last known exposure                  ___/___/___                  Month Day Year</p>	<p><b>4. Location of exposure</b></p> <p>Reporting state _____</p> <p>If multiple states involved:  <input type="checkbox"/> Exposure occurred in multiple states  <input type="checkbox"/> Exposure occurred in single state, but cases resided in multiple states                  Other states: _____</p> <p>Reporting county _____</p> <p>If multiple counties involved:  <input type="checkbox"/> Exposure occurred in multiple counties  <input type="checkbox"/> Exposure occurred in one county, but cases resided in multiple counties                  Other counties: _____</p>
<p><b>2. Number of cases</b></p> <p>Lab-confirmed cases _____(A)                  Including _____ secondary cases</p> <p>Probable cases _____(B)                  Including _____ secondary cases</p> <p>Estimated total ill _____                  (if greater than sum A + B)</p>		

<p><b>5. Approximate percentage of cases in each age group</b></p> <p>&lt;1 year ___%    20–49 yrs ___%                  1–4 yrs ___%    50 yrs ___%                  5–19 yrs ___%    Unknown ___%</p>	<p><b>6. Sex</b>                  (estimated percentage of the total cases)</p> <p>Male ___%                  Female ___%</p>	<p><b>7. Investigation methods</b> (check all that apply)</p> <p><input type="checkbox"/> Interviews of only cases                      <input type="checkbox"/> Environment / food sample cultures  <input type="checkbox"/> Food preparation review                      <input type="checkbox"/> Food product traceback  <input type="checkbox"/> Investigation at factory or production plant    <input type="checkbox"/> Case-control study  <input type="checkbox"/> Investigation at original source (farm, marine estuary, etc.)    <input type="checkbox"/> Cohort study</p>
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**8. Implicated food(s)** (please provide known information)

Name of food e.g. lasagne	Main ingredient(s) e.g. pasta, sauce, eggs, beef	Contaminated ingredient(s) e.g. eggs	Reason(s) suspected (see codes just below) e.g. 4	Method of preparation (see attached codes) e.g. M1
1)				
2)				
3)				

Food vehicle undetermined

*Reason suspected* (list above all that apply)

1. Statistical evidence from epidemiological investigation	4. Other data (e.g. same phage type found on farm that supplied eggs)
2. Laboratory evidence (e.g. identification of agent in food)	5. Specific evidence lacking but prior experience makes it likely source
3. Compelling supportive information	

**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 MMWR2000/Vol. 49/SS-1/App. B)

Etiology		Serotype	Other characteristics (e.g. phage type)	Detected in (see codes just below)
1)	<input type="checkbox"/> Confirmed			
2)	<input type="checkbox"/> Confirmed			
3)	<input type="checkbox"/> Confirmed			

Etiology undetermined

*Detected in* (list above all that apply)  
 1. Patient specimen(s) 2. Food specimen(s) 3. Environment specimen(s) 4. Food worker specimen(s)

10. Isolate subtype	State Lab. ID	PFGE (PulseNet designation)	PFGE (PulseNet designation)
1)			
2)			
3)			

**11. Contributing factors** (check all that apply: see attached codes and explanations)

Contributing factors unknown

**Contamination factor**  
 C1  C2  C3  C4  C5  C6  C7  C8  C9  C10  C11  C12  C13  C14  C15 (*describe in Comments*)  N/A

**Proliferation/amplification factor (bacterial outbreaks only)**  
 P1  P2  P3  P4  P5  P6  P7  P8  P9  P10  P11  P12 (*describe in Comments*)  N/A

**Survival factor (microbial outbreaks only)**  
 S1  S2  S3  S4  S5 (*describe in Comments*)  N/A

**Was food-worker implicated as the source of contamination?**  Yes  No  
 If yes, please check **only one** of following:  
 laboratory *and* epidemiologic evidence  
 epidemiologic evidence (*w/o* lab confirmation)  
 lab evidence (*w/o* epidemiologic evidence)  
 prior experience makes this the likely source (*please explain in Comments*)

Part 2: Additional information			
<b>12. Symptoms, signs and outcomes</b>			<b>13. Incubation period</b> (circle appropriate units) Shortest _____ (hours, days) Longest _____ (hours, days) Median _____ (hours, days) <input type="checkbox"/> Unknown
<b>Feature</b>	<b>Cases with outcome/feature</b>	<b>Total cases for whom you have information available</b>	
Healthcare provider visit			<b>14. Duration of Illness</b> (among those who recovered) (circle appropriate units) Shortest _____ (hours, days) Longest _____ (hours, days) Median _____ (hours, days) <input type="checkbox"/> Unknown
Hospitalization			
Death			* Use the following terms, if appropriate, to describe other common characteristics of cases: Anaphylaxis                      Headache                      Tachycardia Arthralgia                          Hypotension                    Temperature reversal Bradycardia                        Itching                          Thrombocytopenia Bullous skin lesions              Jaundice                        Urticaria Coma                                    Lethargy                        Wheezing Cough                                    Myalgia Descending paralysis              Paraesthesia Diplopia                                Septicaemia Flushing                                Sore throat
Vomiting			
Diarrhoea			
Bloody stools			
Fever			
Abdominal cramps			
HUS or TTP			
Asymptomatic			
*			
*			
*			

**15. If cohort investigation conducted:**

Attack rate\* =  $\frac{\text{Exposed and ill}}{\text{Total number exposed for whom you have illness information}} \times 100 = \text{\_\_\_\_\_\%}$

\* The attack rate is applied to persons in a cohort who were exposed to the implicated vehicle. The numerator is the number of persons who were exposed and became ill; the denominator is the total number of persons exposed to the implicated vehicle. If the vehicle is unknown, then the attack rate should not be calculated.

**16. Location where food was prepared**

(check all that apply)

- Restaurant or deli
  - Day care center
  - School
  - Office setting
  - Workplace cafeteria
  - Banquet facility
  - Picnic
  - Caterer
  - Grocery store
  - Fair, festival, other temporary/ mobile services
  - Commercial product, served without further preparation
  - Unknown or undetermined
  - Other (describe) \_\_\_\_\_
- Nursing home
  - Prison, jail
  - Private home
  - Workplace, not cafeteria
  - Wedding reception
  - Church, temple, etc.
  - Camp
  - Contaminated food imported into U.S.
  - Hospital

**17. Location of exposure or where food was eaten**

(check all that apply)

- Restaurant or deli
  - Day care center
  - School
  - Office setting
  - Workplace cafeteria
  - Banquet facility
  - Picnic
  - Grocery store
  - Fair, festival, temporary/ mobile service
  - Unknown or undetermined
  - Other (describe) \_\_\_\_\_
- Nursing home
  - Prison, jail
  - Private home
  - Workplace, not cafeteria
  - Wedding reception
  - Church, temple, etc.
  - Camp
  - Hospital

**18. Trace back**

Please check if trace back conducted.

Source to which trace back led: \_\_\_\_\_

Source (e.g. chicken farm, tomato processing plant)	Location of source		Comments
	State	County	

**19. Recall**

Please check if any food product recalled.

Recall comments

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**20. Available reports** (please attach)

- Unpublished agency report
- Epi-Aid report
- Publication (please reference if not attached)

\_\_\_\_\_

\_\_\_\_\_

**21. Agency reporting this outbreak**

\_\_\_\_\_

**Contact person:**

Name \_\_\_\_\_

Title \_\_\_\_\_

Phone \_\_\_\_\_

Fax \_\_\_\_\_

E-mail \_\_\_\_\_

**22. Remarks**

Briefly describe important aspects of the outbreak not covered above (e.g. restaurant closure, immunoglobulin administration, economic impact, etc.)

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_



**Part 3: School questions**

**1. Did the outbreak involve a single or multiple schools?**

- Single
- Multiple (if yes, number of schools \_\_\_\_)

**2. School characteristics** (for all involved students in all involved schools)

- a) Total approximate enrolment  
 \_\_\_\_ (number of students)  
 Unknown or undetermined
- b) Grade level(s) (please check all grades affected)  
 Preschool  
 Grade school (grades K-12)  
 Please check all grades affected:  K  1st  2nd  3rd  4th  5th  6th  7th  8th  9th  10th  11th  12th  
 College/university/technical school  
 Unknown or undetermined
- c) Primary funding of involved school(s)  
 Public  Private  Unknown or undetermined

**3. Describe the preparation of the implicated item:**

- Heat and serve (item mostly prepared or cooked off-site, reheated on-site)
- Served a-la-carte
- Serve only (preheated or served cold)
- Cooked on-site using primary ingredients
- Provided by a food service management company
- Provided by a fast food vendor
- Provided by a pre-plate company
- Part of a club/fundraising event
- Made in the classroom
- Brought by a student/teacher/parent
- Other \_\_\_\_\_
- Unknown or undetermined

**4. How many times has the state, county or local health department inspected this school cafeteria or kitchen in the 12 months before the outbreak?\***

- Once
- Twice
- More than two times
- Not inspected
- Unknown or undetermined

\*If there are multiple schools involved, please answer according to the most affected school.

**5. Does the school have a HACCP plan in place for the school feeding program?\***

- Yes
- No
- Unknown or undetermined

\*If there are multiple schools involved, please answer according to the most affected school.

**6. Was implicated food item provided to the school through the National School Lunch/Breakfast Program?**

- Yes
- No
- Unknown or undetermined

**If Yes, was the implicated food item donated/purchased by :**

- USDA through the Commodity Distribution Program
- Purchased commercially by the state/school authority
- Other \_\_\_\_\_
- Unknown or undetermined

#### 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 by the retailer)
  - 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 *E. coli* or *Salmonella* enteritidis only)

##### 1. Mode of transmission (for greater than 50% of cases)

Select one:

- Food
- Person to person
- Swimming or recreational water
- Drinking water
- Contact with animals or their environment
- Unknown or undetermined

#### Part 6: Additional egg questions

##### 1. Were eggs (check all that apply):

- in-shell, un-pasteurized?
- in-shell, pasteurized?
- liquid or dry egg product?
- stored with inadequate refrigeration during or after sale?
- consumed raw?
- consumed undercooked?
- pooled?

##### 2. If eggs traced back to farm, was *Salmonella* enteritidis found on the farm?

- Yes
- No
- Unknown or undetermined

Comment: \_\_\_\_\_  
\_\_\_\_\_

### Contamination factors:<sup>1</sup>

- C1 – Toxic substance part of tissue (e.g. ciguatera)
- C2 – Poisonous substance intentionally added (e.g. cyanide or phenolphthalein added to cause illness)
- C3 – Poisonous or physical substance accidentally/incidentally added (e.g. sanitizer or cleaning compound)
- C4 – Addition of excessive quantities of ingredients that are toxic under these situations (e.g. niacin poisoning in bread)
- C5 – Toxic container or pipelines (e.g. galvanized containers with acid food, copper pipe with carbonated beverages)
- C6 – Raw product/ingredient contaminated by pathogens from animal or environment (e.g. *Salmonella* enteritidis in egg, norovirus in shellfish, *E. coli* in sprouts)
- C7 – Ingestion of contaminated raw products (e.g. raw shellfish, produce, eggs)
- C8 – Obtaining foods from polluted sources (e.g. shellfish)
- C9 – Cross-contamination from raw ingredient of animal origin (e.g. raw poultry on the cutting board)
- C10 – Bare-handed contact by handler/worker/preparer (e.g. with ready-to-eat food)
- C11 – Glove-handed contact by handler/worker/preparer (e.g. with ready-to-eat food)
- C12 – Handling by an infected person or carrier of pathogen (e.g. *Staphylococcus*, *Salmonella*, norovirus)
- C13 – Inadequate cleaning of processing/preparation equipment/utensils leads to contamination of vehicle (e.g. cutting boards)
- C14 – Storage in contaminated environment leads to contamination of vehicle (e.g. store room, refrigerator)
- C15 – Other source of contamination (*please describe in Comments*)

### Proliferation/amplification factors:<sup>1</sup>

- P1 – Allowing foods to remain at room or warm outdoor temperature for several hours (e.g. during preparation or holding for service)
- P2 – Slow cooling (e.g. deep containers or large roasts)
- P3 – Inadequate cold-holding temperatures (e.g. refrigerator inadequate/not working, iced holding inadequate)
- P4 – Preparing foods a half day or more before serving (e.g. banquet preparation a day in advance)
- P5 – Prolonged cold storage for several weeks (e.g. permits slow growth of psychrophilic pathogens)
- P6 – Insufficient time and/or temperature during hot holding (e.g. malfunctioning equipment, too large a mass of food)
- P7 – Insufficient acidification (e.g. home canned foods)
- P8 – Insufficiently low water activity (e.g. smoked/salted fish)
- P9 – Inadequate thawing of frozen products (e.g. room thawing)
- P10 – Anaerobic packaging/modified atmosphere (e.g. vacuum packed fish, salad in gas flushed bag)
- P11 – Inadequate fermentation (e.g. processed meat, cheese)
- P12 – Other situations that promote or allow microbial growth or toxic production (*please describe in Comments*)

### Survival factors:<sup>1</sup>

- S1 – Insufficient time and/or temperature during initial cooking/heat processing (e.g. roasted meats/poultry, canned foods, pasteurization)
- S2 – Insufficient time and/or temperature during reheating (e.g. sauces, roasts)
- S3 – Inadequate acidification (e.g. mayonnaise, tomatoes canned)
- S4 – Insufficient thawing, followed by insufficient cooking (e.g. frozen turkey)
- S5 – Other process failures that permit the agent to survive (*please describe in Comments*)

### Method of preparation:<sup>2</sup>

- M1 – Foods eaten raw or lightly cooked (e.g. hard shell clams, sunny side up eggs)
- M2 – Solid masses of potentially hazardous foods (e.g. casseroles, lasagna, stuffing)
- M3 – Multiple foods (e.g. smorgasbord, buffet)
- M4 – Cook/serve foods (e.g. steak, fish fillet)
- M5 – Natural toxicant (e.g. poisonous mushrooms, paralytic shellfish poisoning)
- M6 – Roasted meat/poultry (e.g. roast beef, roast turkey)
- M7 – Salads prepared with one or more cooked ingredients (e.g. macaroni, potato, tuna)
- M8 – Liquid or semi-solid mixtures of potentially hazardous foods (e.g. gravy, chili, sauce)
- M9 – Chemical contamination (e.g. heavy metal, pesticide)
- M10 – Baked goods (e.g. pies, eclairs)
- M11 – Commercially processed foods (e.g. canned fruits and vegetables, ice cream)
- M12 – Sandwiches (e.g. hot dog, hamburger, Monte Cristo)
- M13 – Beverages (e.g. carbonated and non-carbonated, milk)
- M14 – Salads with raw ingredients (e.g. green salad, fruit salad)
- M15 – Other, does not fit into above categories (*please describe in Comments*)
- M16 – Unknown, vehicle was not identified

<sup>1</sup> Bryan FL, Guzewich JJ, Todd ECD. Surveillance of foodborne disease. III. Summary and presentation of data on vehicles and contributory factors: their value and limitations. *Journal of Food Protection*, 1997, 60(6):701–714.

<sup>2</sup> Weingold SE, Guzewich JJ, Fudala JK. Use of foodborne disease data for HACCP risk assessment. *Journal of Food Protection*, 1994, 57(9):820–830.

