The Role Of The Laboratory In The Diagnosis & Management Of Foodborne Pathogens
Listeria monocytogenes

WHO-NHLS-NICD Listeriosis Incident Management Team
24-25 April 2018

With much gratitude to Shirley du Plessis

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Listeria in foods

- Pirie, Harvey: bacterium isolated from a gerbil in South Africa named *Listerella hepatolytica*
- Later renamed *Listeria monocytogenes*
- ‘Awkward and unnoticed adolescent’ emerged into an important foodborne pathogen
- 1981 discovery that it could be transmitted by foods
- 1985 identified as the causative agent in a large (142 cases with 48 deaths) outbreak associated with a Mexican-style soft cheese
- Today *L monocytogenes* a source of continual problems for the food industry and regulatory agencies

Listeria

- 6 species
  - ***L monocytogenes***
  - *L innocua, L welshimeri, L seeligeri, L ivanovii, L grayi* and 4 subspecies
    - Presence of *Listeria* spp in a food manufacturing environment is often used as an indicator for *L monocytogenes* infections
  - May form difficult-to-remove biofilms
  - Growth over wide temperature range from 5°C- 60°C, best at 25°C
- Infective dose in vulnerable individuals can be very low (100 CFU/g)
- Transmission: foodborne; trans-placental; person-to-person transmission rare
- Ubiquitous: soil, plant, animal, fish & other marine foods

Listeria

- Found in a variety of food products but hazardous in chilled processed foods with a long shelf-life (e.g. smoked fish, pâtés, soft cheeses & ready-to-eat cooked meats)

- Can grow slowly at temperatures close to 0°C and can multiply to dangerous levels in refrigerated foods unless controlled

- Legislation is introduced in many countries e.g.
  - in EU ready-to-eat foods: regular testing required with limit of absent in 25g at point of production and up to 100 CFU/g at the end of shelf life
  - in USA, regular testing and a ‘zero tolerance’ in ready-to-eat foods

Limiting Parameters for Growth of *Listeria monocytogenes*

Parameter	*Minimum Conditions Allowing Growth*	*Reference*
Temperature	0.1 to -0.4° C	515,516
	1.1° C	263
pH*	5.0 to 5.7; 4° C 4.3 to 5.2; 30° C	149a
	4.4 to 5.0; 10° C 4.4 to 5.2; 25° C	473a
	5.23; 4° C 4.62; r C 4.62; 10° C 4.59; 20° C 4.39; 30° C	189a
Water activity	4° C, 10° C; 0.93-NaCl 15° C; 0.91-glycerol, NaCl 30° C; 0.90-glycerol 40° C; 0.93-glycerol	151a
	4° C 0.92-glycerol 0.93-0.96-sucrose 0.94-NaCl	485a
	30° C 0.90-glycerol 0.92 to 0.96-sucrose 0.92-NaCl	485a
	21° C 0.924-NaCl 0.925-sucrose 0.911-glycerol	377a

* Values vary depending on acidulant used

Generation Times of *Listeria monocytogenes* in Foods

Food	Temperature (°C)	Generation Time (h)	Reference
Milk	4	25	150
	9.5	11	57
	10	10.8	150
Ice cream	4	21.6	437
	9.5	11	57
	21	1.1	437
Chicken broth	2.5	35	516
	9.3	6	516
Crawfish tail meat	0	72.2	120
	6	17.0	120
	12	6.9	120
Roast beef	-1.5	129.0	241
	3	37.6	241
Corned beef	0	110.0	208
Ham	5	33.2	208
	10	13.4	208
	15	6.1	208
Cauliflower	15	7.2	29

Listeriosis: 2017-18 RSA outbreak

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

Situational Report			
Outbreak Name	Listeriosis	**Country affected**	South Africa
Date & Time of report	20 April 2018	**Investigation start date**	August 2017
Prepared by	National Listeria Incident Management Team (IMT)		

Figure 1 (left). Electron micrograph showing two *Listeria monocytogenes* bacteria, illustrating the single 'flagellum' that makes the organism motile (courtesy Dr Monica Birkhead, NICD)

1. HIGHLIGHTS

- A multisectorial incident management team (IMT) has been formed by the National Department of Health (NDoH) to strengthen co-ordination of outbreak response and strengthen health systems to prevent future outbreaks. Funding is being sourced.
- The number of cases of laboratory-confirmed listeriosis reported per week has decreased since the implicated products were recalled on 04 March 2018, with eight additional cases reported this week. Of the eight cases reported this week, one case occurred in October 2017 and was retrospectively reported.
- Since the recall, a total of 50 cases have been reported
    a. Twenty-four (48%) were among neonates ≤28 days old
    b. Three (6%) were among children age 1 month to 14 years old
- As of 17 April 2018, a total of 1 019 laboratory-confirmed listeriosis cases have been reported to NICD since 01 January 2017.

Bacteriological Aspects of Recent Food-Poisoning Outbreaks on the Witwatersrand

AN ACUTE OUTBREAK OF STAPHYLOCOCCAL ENTEROTOXIN FOOD POISONING

EDMUND D. COOPER, M.D., F.R.F.P.S. (GLASG.), D.P.H., F.R.S.H., *Medical Officer of Health, City of Cape Town*

AN OUTBREAK OF FOOD POISONING AMONG CHILDREN ATTENDING AN INTERNATIONAL SPORTS EVENT IN JOHANNESBURG

J Andreas Karas, Mark P Nicol, Neil Martinson, Robin Heubner

Outbreaks of food-borne disease – a common occurrence but rarely reported

Anthony M Smith, Anne-Marie Gouws, Greta Hoyland, Arvinda Sooka, Karen H Keddy, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA)

Communicable Diseases Communiqué

Volume 8, No. 7

"All Africa Games" – 10th September 1999

SAMJ 2001;91(5):417-421

- Participants included 578 SA children
- Meal eaten at Johannesburg Athletics Stadium btw. 15h00-17h00
  - Either chicken OR stew, together with maize-meal porridge & fruit juice
- Shortly after eating the meal: 513 children taken to 3 hospitals – 149 admitted for observation
- Questionnaires distributed to 578 children; 361 returned (response rate 62.5%)

THE ALL AFRICA GAMES...

- Of 361 children involved, information available
  - vomiting (n=134) - fruit juice
  - diarrhea (n=53) - maize meal porridge, chicken stew

Table IV. Odds ratios of foods consumed that were reported to cause illness

Food	Odds ratio (95% CI)	*P*-value
Fruit juice	19.97 (6.84 - 61.05)	$< 10^{-6}$
Beef stew	2.38 (1.28 - 4.44)	0.003
Chicken stew	1.21 (0.64 - 2.33)	0.53
Maize-meal porridge	5.55 (2.70 - 11.62)	$< 10^{-6}$

CI = confidence interval.

1

- Table shows the odds of developing illness after consumption of the various foods.

2

- Fruit juice (OR=20.0) and maize-meal porridge was strongly associated with the development of any illness.

MULTIVARIATE ANALYSIS OF ASSOCIATION BETWEEN FOODS CONSUMED AND CASE-DEFINITION A (ACUTE NAUSEA OR VOMITING) AND B (DIARRHOEA)

	Case Definition A			*Case Definition B*	
Food	**Odds ratio (95%)**		**p value**	**Odds ratio (95%)**	**p value**
Fruit juice	11.8	(11.5 - 90.4)	0.0177	2.4 (0.3 - 0.9)	0.4187
Beef stew	0.7	(0.3 - 1.5)	0.3064	0.9 (0.2 - 3.2)	0.8164
Chicken stew	1.6	(0.9 - 3.0)	0.1233	7.3 (3.4 - 5.9)	0.0001
Maize-meal porridge	3.0	(0.9 - 3.0)	0.0005	8.8 (2.6 - 0.2)	0.0005

Subjects that satisfied definition B were more likely to have consumed both the maize-meal porridge (OR=8.8) and chicken stew (OR=7.3)

Subjects that satisfied definition A were more likely to have consumed fruit juice (OR=11.8) or the maize-meal porridge (OR=3.0)

Findings

- High bacterial counts (TPC: 1,6 million cfu/ml – should be < 100cfu/ml)
- *Shigella flexneri* – isolated in maize-meal porridge
- Environmental findings & microbiological sampling of foods, utensils & food preparation areas – unacceptable parameters
- High Court of South Africa:" Case 2000/3100, Nise Caterers cc *versus* Times Media Limited" – an interesting surprise

Major factors that impede the successful investigation of food-borne outbreaks

- Often, information about food-consumption of non-affected people not collected
- Delays &/or incorrect collection of implicated food / water samples, delays in their submission to the laboratory, chain of custody not maintained, cold chain (≤ 7^{o} C) not maintained – consequently, legal requirements not met
- Failure to arrange for collection of clinical samples (stools, rectal swabs, vomitus) from affected people
- Psychogenic amplification


ALL AFRICA GAMES, THE CLOSING CEREMONY..
HELLO, CHOREOGRAPHER? .. DOES THIS REPRESENT THE END OF THE GAMES, OR ARE WE STILL USING THE SAME CATERER?

No. R. 328 **20 April 2007**

FOODSTUFFS, COSMETICS AND DISINFECTANTS ACT, 1972
(ACT NO. 54 OF 1972)

REGULATIONS RELATING TO THE POWERS AND DUTIES OF INSPECTORS AND ANALYSTS CONDUCTING INSPECTIONS AND ANALYSES ON FOODSTUFFS AND AT FOOD PREMISES

The Minister of Health has, in terms of section 15(1) of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972), made the regulations in the Schedule.

(2) The inspector shall follow the following procedure when he or she is taking a sample in terms of these regulations with due regard to the powers conferred on inspectors under section 11(1) of the Act:

(a) The inspector shall take a sample in the presence of -

(i) the person in charge; or

(ii) any other adult person who is employed in those premises, as a witness if the person in charge is not present.

(b) The inspector shall, as soon as practicable after the sample has been obtained in terms of paragraph (a)(ii), notify the person in charge, in writing, of the sampling and of the purpose thereof.

SAMPLING STEPS
R. 328 of 2007

1. Sample Collection

✓ Sufficient Quantity in container/packaging -2(1)(f)

✓ Sample in the presence of person in charge/any other adult person

✓ Notification, in writing, of the sampling and purpose

(c) The inspector shall ascertain from the person referred to in paragraph (a) in writing and in a format provided in **Annexure A**, whether a part of such sample for examination or analysis is required and if so, the inspector shall -

(i) divide the sample in such a manner as its nature permits, into three separate parts which shall be as identical as possible. (One of the portions shall be handed to such person, one sent to an analyst for analysis or examination and one carefully kept by the inspector until the case has been finalised);

(ii) if the contents of one package are not sufficient for analysis or examination if divided as aforesaid, obtain additional packages of the property of the same person similarly labelled and purporting to contain a similar article, and shall mix the contents of two or more such packages then and there and divide the mixture and deal with it as provided; and

SAMPLING STEPS
R 328 of 2007

2. Sample Division - Offer in writing **Annexure A** – [Reg 2(c)]

✓ If accepted divide into three identical parts

- Part A- person in charge
- Part B-sent to analyst
- Part C- carefully kept by the inspector until the case has been finalized - RETENTION SAMPLE

✓ Insufficient contents of one package

- obtain additional packages of the same product, mix and divide as above

(iii) pack, seal and label with a special label in a format provided in **Annexure B**, each of the three parts of a sample referred to in subparagraph (i) to indicate its nature, origin and identify it with-

(aa) an identification number;

(bb) concise details regarding the contents;

(cc) the nature of the examination or analysis required;

(dd) the date on which the sample was taken; and

(ee) his or her name and work address.

LABELING, PACKAGING, SEALING (CHAIN OF CUSTODY), TEST REQUEST

(d) If the offer to divide the sample is not accepted, the undivided sample shall be packed, sealed, labelled with a special label to indicate its origin, nature and to identify it as indicated in paragraph (c) (iii) and sent to an analyst for analysis or examination.

(e) In the case of a perishable foodstuff, or a foodstuff in a sealed package, or where the opening of such package would hamper analysis or examination, or where no person referred to in paragraph (c) is present, a similar procedure to that described in paragraph (d) shall be followed.

(f) The original label of the package, if any, or a copy thereof shall accompany the sample sent to the analyst.

(g) In the case of milk or cream sampled for chemical or compositional analysis, the preservative tricresol, may be added. If a person referred to in paragraph (c) is present, the preservative shall be added to the sample in his presence and he shall be informed of the nature of the preservative.

(h) The sample may be delivered to the analyst by any convenient means provided the inspector's seal remains intact.

RECEIVED BY ANALYST WITH INTACT SEAL (CHAIN OF CUSTODY)

SAMPLING STEPS
R 328 of 2007

3. Pack, seal and label each of the three parts - label format provided in **Annexure B.**

(a) an identification number;

(b) concise details regarding the contents;

(c) the nature of the examination or analysis required;

(d) the date on which the sample was taken;

(e) Inspectors name & work address.

SAMPLING STEPS
R 328 of 2007

- ✓ If offer to divide the sample is not accepted – pack, seal & label the undivided sample

- ✓ Perishable foodstuff or foodstuff in a sealed package or where opening of package would hamper analysis or examination will not be divided

- ✓ The original label of the package or a copy - sent to the analyst

i) Samples for bacteriological analysis shall be taken with sterilized equipment and transferred to sterile sample containers taking precautions to prevent the contamination of the samples. The sample container shall be stoppered and, within 15 minutes of the sample being taken, shall be surrounded by crushed ice or other suitable refrigerant which comes into contact with the container and is capable of reducing the temperature of the sample to 7°C and maintaining it at that temperature or below, but not frozen, until delivered to an analyst.

ASEPTIC TECHNIQUE, COLD CHAIN, DELIVERY

(3) In addition to the procedure described in subregulation (2), when sampling of milk and milk products is carried out, the contents of the International Standard: *ISO 707:1997: Milk and Milk Products – Guidance on Sampling*, shall, where applicable, be taken into consideration.

SAMPLING EQUIPMENT FOR BACTERIOLOGICAL ANALYSIS R 328 of 2007

- ✓ Must use **sterilized equipment** to take a sample and transfer to sterile container

- ✓ Surround the sample **within 15 minutes** after sampling with **crushed ice or any other suitable refrigerant T**o reduce the temperature of the sample to **7ºC**

- ✓ Samples must be **maintained at 7ºC or below**, **BUT NOT FROZEN** until it is delivered to the analyst

Duties of Analysts

7. (1) An analyst referred to in **section 12** (2) of the Act shall complete a certificate in a format provided in **Annexure H** of these regulations.

(2) In the case of milk or cream, besides any other aspects which have to be investigated, it shall be determined and reported whether a preservative is present and, if so, whether it is a preservative prescribed by regulation for that purpose.

(3) In the case of a sample of a foodstuff which is not perishable and which is found on analysis or examination to be adulterated or falsely described or otherwise not to comply with the requirements of the Act, and which was not divided by the inspector, the unused portion, if any, of the sample shall be closed, sealed and carefully retained by the analyst until after the conclusion of any prosecution in connection therewith.

DUTIES OF THE ANALYST

SAMPLING STEPS
R 328 of 2007

- ✓ Delivery to analyst by any convenient means BUT provided Inspector's seal remains intact

- ✓ Chemical Samples:
  DoH Forensic Chemical Laboratories (Cape Town & Pretoria)

- ✓ Microbiological Sampling: (NHLS/Private Laboratory)

Sampling

- Adequacy and condition of a sample for examination are of primary importance
- Improper collection, mishandling, non-representative samples of a sampled lot => meaningless laboratory results
- As interpretations about a large consignment of food are based on a relatively small sample, use best food testing equipment for accurate results

Equipment requirements - Items that are NOT provided, and must be sourced by EHPs

- Alcohol-based hand-rub gel (hand hygiene)
- PPE: hand sanitizer, sterile gloves, disposable plastic aprons
- Cooler boxes
- Courier waybills
- Temperature monitoring device
- Frozen cooler bricks
- Pen, marker pens
- 70% alcohol (NOT 100% alcohol, NOT methylated spirits)
- Matches / lighter
- Forceps and other sampling utensils
  - MUST BE WASHED TO REMOVE ADHERING DEBRIS PRIOR TO ANY DISINFECTION / STERILIZATION PROCESS
- Spirit or a gas burner

Equipment requirements - Items that are provided by the NHLS

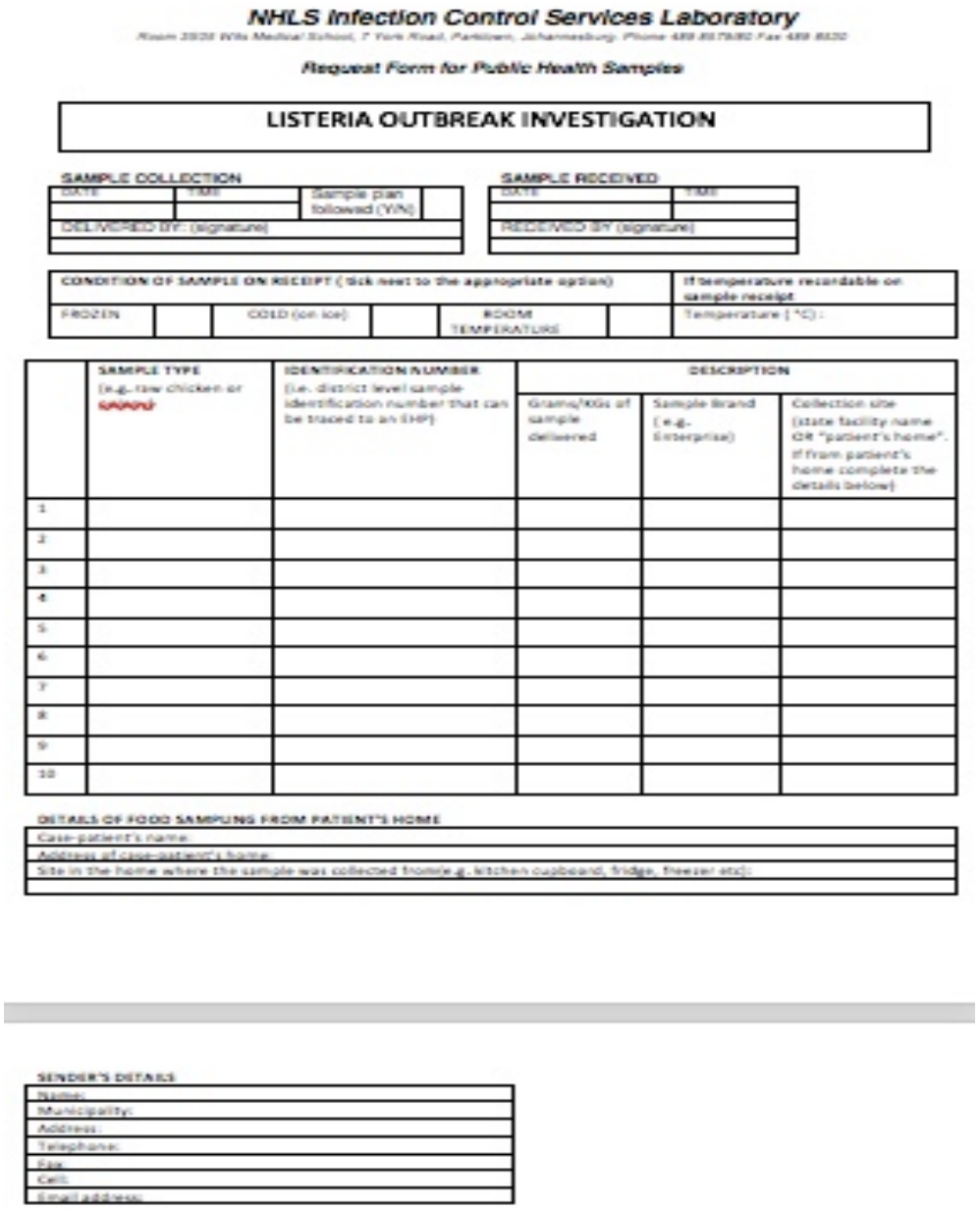
- Equipment provided by NHLS
  - Sample containers (red topped, sealed, and irradiated – states ‘sterile’)
  - Laboratory request forms
  - 10 ml full strength Fraser’s broth in a sterile MacCartney bottle with screw-top
  - Sterile swabs individually packaged from manufacturer

- Procedure to get above items
  - To be confirmed

Sampling: *Listeria* outbreak investigation form

- Laboratory request form ("*Listeria* outbreak investigation form")
- Sample type & details
- Patient details if it is a clinical sample
- Location where samples were taken
- Local authority responsible for payment
- Reference number
- Date taken
- Contact person (person who took the samples)
- Phone, cell, & e-mail address
- Test request

Listeria outbreak investigation form



NHLS Infection Control Services Laboratory

Room 2020 Wits Medical School, 7 York Road, Parktown, Johannesburg. Phone 489 8170/80 Fax 489 8020

Request Form for Public Health Samples

LISTERIA OUTBREAK INVESTIGATION

SAMPLE COLLECTION

DATE	TIME	Sample plan followed (Y/N)	
DELIVERED BY: (signature)			

SAMPLE RECEIVED

DATE	TIME
RECEIVED BY (signature)	

CONDITION OF SAMPLE ON RECEIPT (Tick next to the appropriate option)						If temperature recordable on sample receipt
FROZEN		COLD (on ice)		ROOM TEMPERATURE		Temperature (°C) :

	SAMPLE TYPE (e.g. raw chicken or ~~sprouts~~)	IDENTIFICATION NUMBER (i.e. district level sample identification number that can be traced to an EHP)	DESCRIPTION		
			Grams/Kgs of sample delivered	Sample brand (e.g. Enterprise)	Collection site (state facility name OR "patient's home". If from patient's home complete the details below)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

DETAILS OF FOOD SAMPLING FROM PATIENT'S HOME

Case-patient's name:
Address of case-patient's home:
Site in the home where the sample was collected from(e.g. kitchen cupboard, fridge, freezer etc):

SENDER'S DETAILS

Name:
Municipality:
Address:
Telephone:
Fax:
Cell:
Email address:

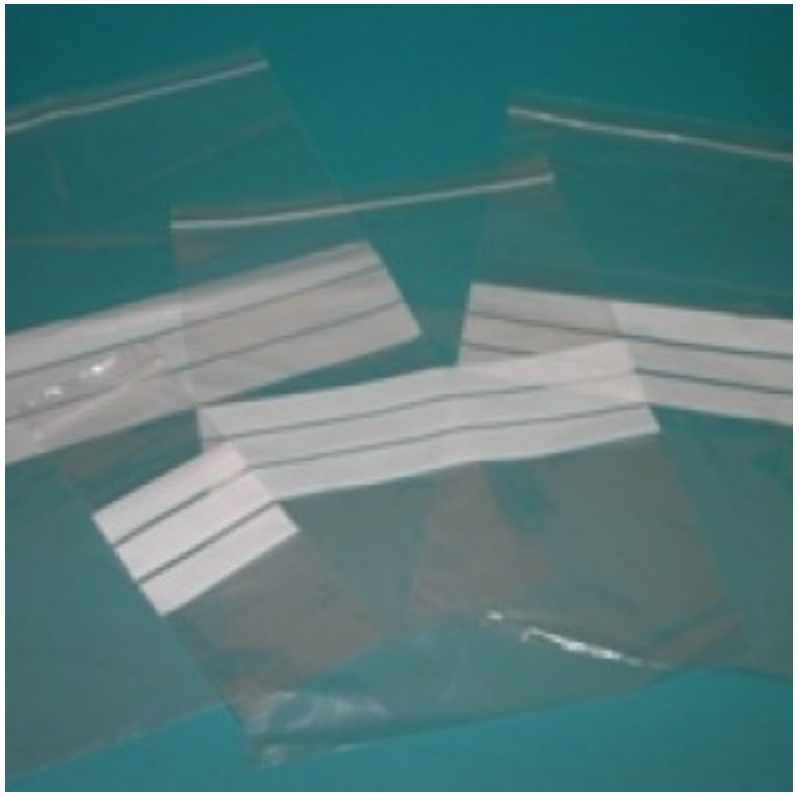
Sampling

- Collecting food samples – ASEPTIC TECHNIQUE
  - Fill in Laboratory request form (see Appendix A)
  - Label sample container before the sample is collected
  - Perform hand hygiene with an alcohol-based Hand-rub
  - Don a disposable plastic apron
  - Don a pair of sterile gloves
  - Use a freshly cleaned & sterilized utensil for each sample
  - Decontaminate with 70% alcohol, allow alcohol to evaporate
  - If utensil heated, wait for the utensil to cool before taking the sample
  - 50 – 100 grams per sample is required (preferably 100 grams)
  - Place the sample into a container, then into a zip-lock and sealed plastic bag & then in the cooler box
- In the event of whole foods, pieces thereof, or meals
  1. Steps 1-5 as above
  2. Place sample (or pieces of a sample) into a (separate) container(s), then into a zip-lock and sealed plastic bag & then in the cooler box

Sampling: hand hygiene

Sampling: utensils

Sampling containers



Sampling

- Taking swab samples from outer packaging of foodstuffs and from the food processing environment
  - Label the 10 ml full strength Fraser's broth (bottle)
  - Select a sterile swab
  - Pre-moisten the swab in the 10 ml full strength Fraser's broth
  - Take the sample with the moistened swab
  - Place the swab back into the 10 ml full strength Fraser's broth and break off the handle
  - Close the 10 ml full strength Fraser's broth lid and place into cooler box

Sampling

- Sample integrity
- All samples should be sealed with tamper-proof tape (very important if one of the parties wishes to pursue legal action or there have been death/s)
- Alternatively, the cooler box may be sealed with tamper-proof tape
- In any event the sample integrity is critical
  - Ensure that samples are not leaking, lids are screwed on properly, samples cannot be broken in transit etc.
  - Do not use glass containers / or polystyrene ‘take-away’ food containers without ensuring that the latter containers are appropriately sealed and placed in a plastic bag

Sampling

- Test Requests (test analysis required)
  - Food for *Listeria*:
    - Culture and Quantification = present or absent plus colony forming units per gram CFU/g
  - Swab for *Listeria*:
    - Culture = present or absent

Sample packaging & transportation

- Place the samples in a sturdy cooler box and not in a cardboard box
- Include enough ice-bricks to maintain a temperature of ≤ 7^{o} C for the full journey
- Include temperature monitoring device in each cooler box
- Samples should not be frozen unless you are sending an already frozen sample
- Sample consignment must be correctly addressed to the testing lab e.g.:
  - NHLS Infection Control Lab, Room 3T09, Wits Medical School, Parktown, Johannesburg (011 489 8579)
- Samples may be sent by Courier (preferable) / sent via the nearest NHLS laboratory (please check that overnight delivery will be made)
- If you are delivering the samples then please ask for directions prior to sampling visit
- Samples must be delivered on the same day they are taken otherwise they must be sent by courier to arrive on the next day
- Should there be an unavoidable delay in sample submission (not ideal!), please refrigerate at a temperature of 1-4°C
- Delay in sending samples may result in microbial overgrowth and/or false negative results
- Samples should not be collected before a long weekend, weekend or Public Holiday to prevent delays in delivery to the laboratory

Sampling packaging

CRĒDO CUBE™ SERIES 4

Superior thermal protection in a convenient mid-size container.

The Crēdo Cube™ is a passive and reusable shipper qualified to hold chilled medical materials at a safe temperature for up to five days. This system is ideal for reducing shipping costs or for circumstances in which unexpected delays may occur.

Modular TIC™ (Thermal Isolation Chamber) walls with integrated PCM (Phase-Change Material) and VIPs (Vacuum Insulated Panels) makes conditioning in a freezer for year-round shipping easy and allows effortless packing and storage.

CRĒDO CUBE™

DURATION
96 - 168 Hours

SERIES 4
(2°C to 8°C)

VOLUME
3L, 4L, 8L, 10L, 12L, 16L, 28L, 42L, 56L, 96L

Sampling packaging

ORCA

2-8°C, 15-25°C, 20-24°C, -20°C

TEMPERATURE CONTROLLED

Food & environmental test sampling procedures

- Environmental sampling and sampling of outer packaging: cotton-tipped swabs premoistened with full strength Fraser's broth (enrichment medium)
  - Qualitative analysis: *Listeria monocytogenes* detected or not
- Food samples, esp. RTE foods: 5, 5g sampling units (portions) for detection & quantitation (enumeration) of *Listeria monocytogenes* PLUS a qualitative cotton tipped swab of internal creases at both ends of plastic wrap packaging

NHLS PH testing laboratory capacity

- April 2018: NHLS PH IPC Laboratory, third floor, Wits Medical School, Parktown, Johannesburg
  - 20-30 food samples for *Listeria monocytogenes* enumerative microbiology per day, with specimen submissions on Tuesday, Wednesday & Thursdays
  - Samples stored in a specially dedicated and locked fridge
  - Environmental swabs: as per sampling requirements [within reason, need approximate numbers]
  - SANAS ISO 17025 accreditation in October 2018

- As from 7 May 2018: NHLS Prince Street PH Laboratory, Durban
  - 20-30 food samples for *Listeria monocytogenes* enumerative microbiology per day, with specimen submissions on Tuesday, Wednesday & Thursdays
  - Environmental swabs: as per sampling requirements

- Activation of NHLS PH Laboratory, Greenpoint, Cape Town planned. Date of commencement of

Sampling: rejection criteria discuss

- Leaky specimens
- Temperature not recordable, or not recorded, or unacceptably high (> 8°C) upon sample receipt by the analyst's receiving office
- Inspector's seal is broken (suggests tampering)
- If multiple products, not from the same source, are in contact with each other
- Dry swab received that has not been put in full strength Fraser's broth

THANK YOU