

## Critical Steps of PT Program in Food Microbiology



*Boonmee Kanjanopat*  
*Betagro Science Center*

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### Steps of PT program in food microbiology

- PT plan
- Sample preparation
- Sample verification
- Sample dispatch
- Reporting the laboratory results to PTP
- Data analysis and performance assessment
- Sending the summary and final report to PP

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**□ Choice of sample matrices on microbiological test**

- ISO 16140-2:2016 has been classified 19 food categories
- PT providers currently make an effort to provide sample matrices in order to fulfill a variety of food categories.

□ Choice of sample matrices on microbiological test

➤ Major sample matrices on microbiological tests

- Meat & Poultry meat
- Seafood
- Dried cereals & vegetable
- Potable water
- Swab sample (Environmental samples)
- Feedstuff




❑ **Main microorganisms used in PT scheme**

- Foodborne pathogens  
e.g. *Salmonella* spp., *Listeria monocytogenes*,  
*Staphylococcus aureus*, *Vibrio parahaemolyticus*
- Waterborne pathogens  
e.g. *Escherichia coli*, *Salmonella* spp.

❑ Main microorganisms used in PT scheme


- Hygiene indicator microorganisms  
e.g. Coliforms, Enterococci
- Microorganisms or group of microorganisms defined in food hygiene or public health criteria

Critical Steps of PT Program in Food Microbiology 

**❑ Type of testing and assessment in microbiological program**

Type of testing	Assessment	Criteria
Qualitative test (Detection test)	Compare to assigned value	Matching : Satisfactory
		Mismatching : Unsatisfactory
Quantitative test (Enumeration test)	z (z´) score	$ z  \leq 2$ : Satisfactory
		$2 <  z  < 3$ : Questionable
		$ z  \geq 3$ : Unsatisfactory

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Critical Steps of PT Program in Food Microbiology 

**❑ Sample preparation : The critical step of PT program for microbiological tests**

➤ **Sample design**

- Real sample, e.g. feed stuff, grain, oatmeal
  - Sample is probably from more than one source
  - Available for some tests, e.g. aerobic plate count, mold count

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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ **Sample design**

- **Simulated sample**

- Samples are spiked with microorganisms and freeze dried.
- Sample matrix is from food raw material e.g. chicken meat, beef, shrimp, fish, oatmeal, milk



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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ **Microorganism design**

- **Target organisms**

- Sample for detection method
  - The target organism is normally foodborne pathogen, e.g. LMO, *Salmonella* spp., *Campylobacter* spp.
  - The target level shall be provided at level likely to cause hazard to human health
  - The target organisms should be sufficiently low level to challenge laboratory performance and the method selected

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- ❑ Sample preparation : The critical step of PT procedure for microbiological tests

➤ **Microorganism design**

- **Target organisms**

- Sample for enumeration method
  - The sample may have a single or a group of target organisms, e.g. *Escherichia coli*, *Staphylococcus aureus*, Coliforms, APC
  - The target organisms shall be provided nearby level specified in microbiological criteria
  - The target level should be used over the limit of quantitation of routine method

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- ❑ Sample preparation : The critical step of PT procedure for microbiological tests

➤ **Microorganism design**

- **Background organisms**

- Naturally or artificially contaminated samples
- Some strains used to simulate background flora may be close to target strain
- Sample for detection method, the background level is normally higher than the target level

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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ Cryopreservative medium (CPM)

- Most of PT samples for microbiological tests are freeze dried form
- CPM is the most important factor for microbial preservation in freeze dried form
- What is the best CPM to keep survival ?

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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ Cryopreservative medium (CPM)

- No one CPM works for all organisms
- Trials and knowledge studies
- Technique of freeze drying probably effects to organism survival



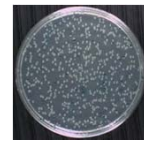
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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ **Sample verification**

- **Real sample**
  - Check the quantity of target organisms after blending
- **Freeze dried sample**
  - Check the recovery of target and background organisms
  - Check contaminants other than organism designed



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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ **Sample verification**

- **Sample homogeneity : factors effect to homogeneity**
  - Inoculum and matrix mixing
  - Consistency of sample dispensed into container
  - Overall dispensing time



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- Sample preparation : The critical step of PT procedure for microbiological tests

➤ Sample verification

- Sample stability : factors effect to stability

- Sample container, e.g. vial, bottle, pouch, petri dish (plate)
- Moisture
- Temperature on storage
- Temperature on transportation & delivery time, i.e. cool pack, not cool



Critical steps of PT program in food microbiology

□ **Problems occasionally found in PT programs**

- Microorganism survival
  - Microbial strains  
e.g. *Bacillus cereus*, *Staph. aureus*,  
*Campylobacter jejuni*
  - Cryopreservative substances
- Sample delivery
  - Ambient & cold temperature

**APFAN PT-2 Workshop**  
Food Analysis Workshop: Proficiency Testing and Reference Materials Development



□ Problems occasionally found in PT rounds

➤ Participant result data

- Low number of participants
- Distribution of participant results
- Blundered results
- Effect to statistical values when the assigned value is derived from consensus values, i.e. robust SD, std. uncertainty of assigned value




**Qualitative test (Detection)**

Sample matrices	Test items	Test unit	Program	n	Assessment	
					S	U
FDC	<i>Listeria monocytogenes</i>	P/A 25 g	M1803	9	9	-
FDC	<i>Salmonella</i> spp.	P/A 25 g	M1808	7	7	-
FDC	<i>Clostridium perfringens</i>	P/A in 0.2 g	M1806	10	10	-
Water (Lyo. culture)	<i>Salmonella</i> spp.	P/A in 100 ml	M1814	15	14	1
Swab (Plastic plate)	<i>Listeria</i> spp.	P/A in swab	M1801	6	6	-
Swab (Plastic plate)	<i>Salmonella</i> spp.	P/A in swab	M1805	7	7	-
Animal feed	<i>Salmonella</i> spp.	P/A 25 g	M1813	13	13	-
Animal feed	<i>Salmonella</i> spp.	P/A in 25 g.	M1815	12	12	-

FDC : Freeze dried chicken , S : Satisfactory , U : Unsatisfactory

**APFAN PT-2 Workshop**  
Food Analysis Workshop: Proficiency Testing and Reference Materials Development




**Quantitative test (Enumeration)**

Sample matrices	Test items	Test unit	Program	n	z (z') score			Blunder r
					$ z  \leq 2$	$2 <  z  < 3$	$ z  \geq 3$	
FDC	Aerobic plate count	cfu/g.	M1804	10	10	-	-	-
FDC	Coliforms	cfu/g.	M1809	8	8	-	-	-
	<i>E.coli</i>	cfu/g.		8	8	-	-	-
FDC	<i>Staphylococcus aureus</i>	cfu/g.	M1810	10	10	-	-	-
Water (Lyo. culture)	Coliforms	MPN/100 ml.	M1802	15	11	2	2	-
	<i>E.coli</i>	MPN/100 ml.		15	11	-	1	3
Water (Lyo. culture)	Aerobic plate count at 35°C	cfu/g.	M1811	14	14	-	-	-
Swab (Plastic plate)	Aerobic plate count	cfu/g.	M1807	13	11	2	-	-
Lyophilized culture	<i>Clostridium perfringens</i>	cfu/g.	M1816	8	7	-	1	-
Animal feed	Aerobic plate count	cfu/g.	M1812	12	12	-	-	-

FDC : Freeze dried chicken

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**Blundered results**

Lab No.	Result (x)	log x	z score
6	<2	-	-
9	<1.1	-	-
15	>1600	-	-

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- ❑ **Summary of critical steps of PT program in food microbiology**
  - **Sample preparation**
    - Microorganism design
    - Cryopreservative medium
    - Recovery of target and non-target organisms
    - Sample homogeneity
    - Sample stability
    - Sample packaging

- ❑ Summary of critical steps of PT program in food microbiology
  - **Problems occasionally found for PT program in food microbiology**
    - Microorganism survival (Sample preparation)
    - Sample delivery
    - Participant result data



**Thank You**

