



Proficiency Testing in Microbiology

Food Analysis Workshop: Proficiency Testing And Reference Materials Development, Bangkok
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Outline of Presentation

- 01 Microbiology Section
- 02 PT in Microbiology
- 03 Bacterial Culture Collections
- 04 Challenges
- 05 Future directions

Microbiology laboratory




Protecting people's health by ensuring that food and water consumed by public are safe and do not pose a threat to health



- ☑ National Reference Laboratory
- ☑ Microbiology testing for food and water samples
- ☑ PT subcontractor for microbiology testing
- ☑ Training and consultation

Capabilities



ISO17025 Accredited Methods

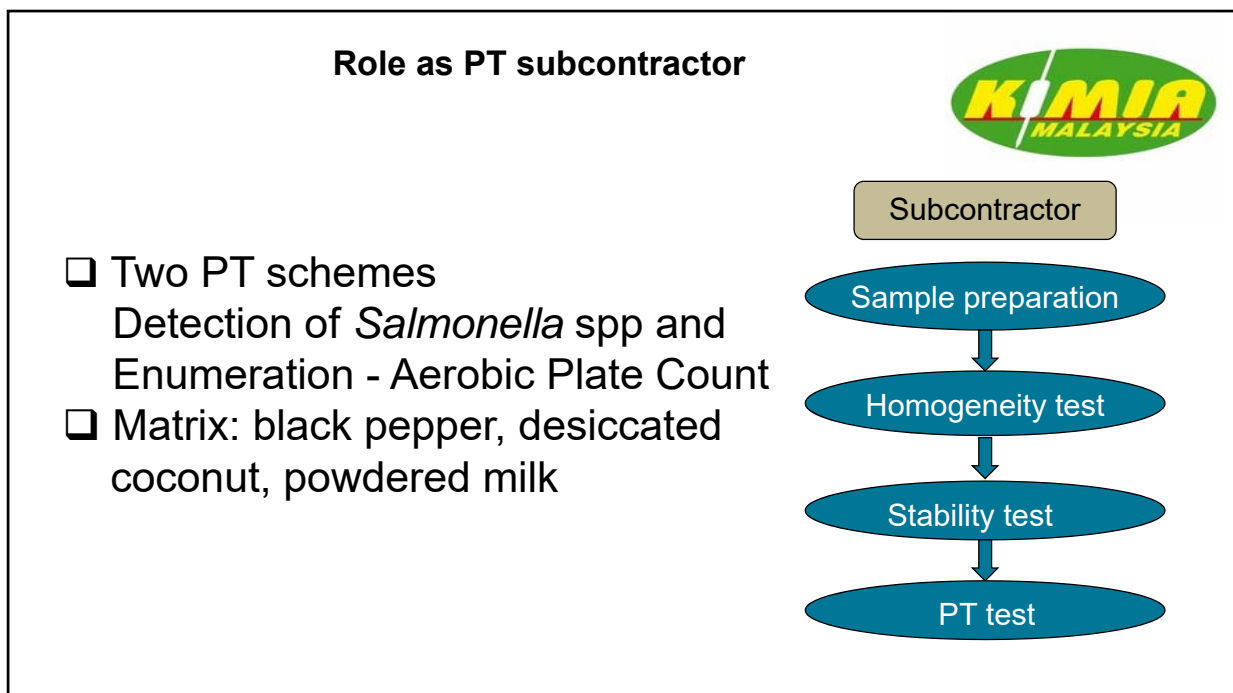
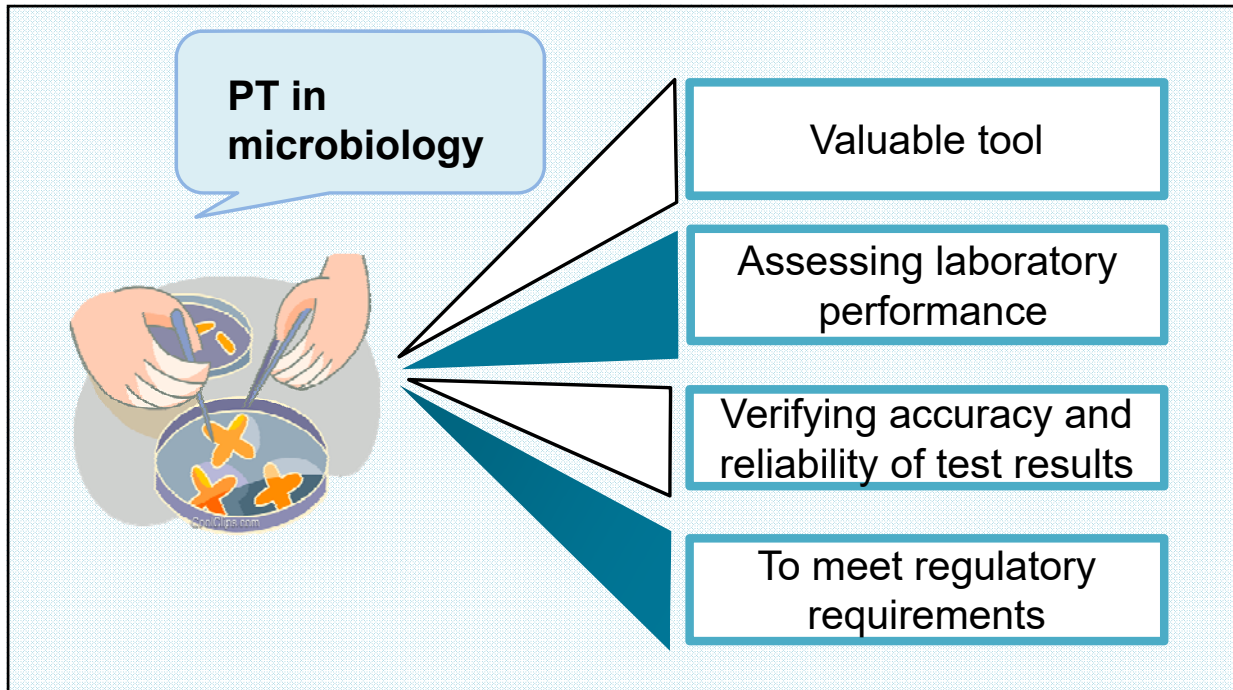
- Method development for *Leptospira* spp and algae (*Cynobacteria*) in recreational and marine water
- Identification of bacteria for Ballast Water Management

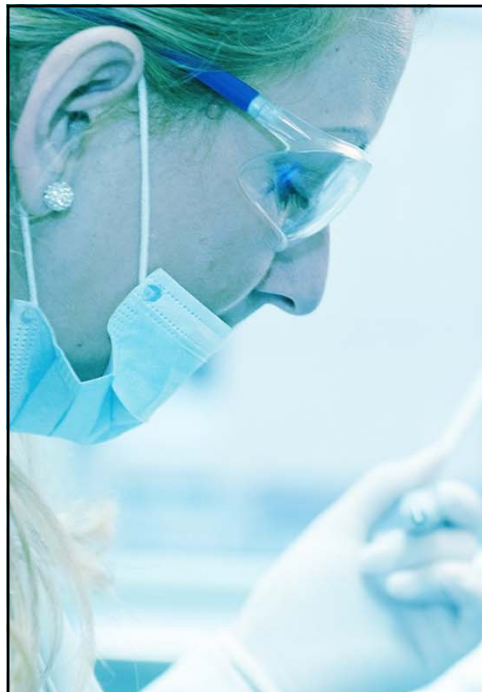
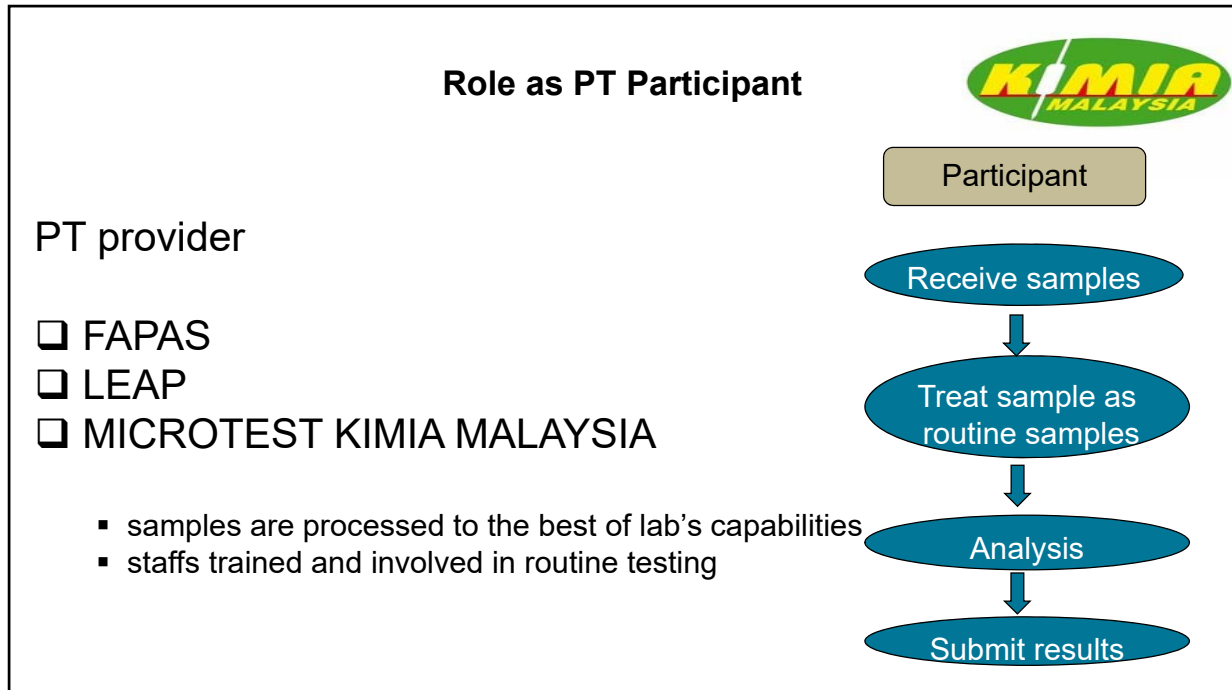
Conventional microbiology

- 19 for water microbiology
- 21 for food microbiology

Molecular DNA

- 19 for food & water microbiology





Bacterial cultures

- authentic biological material for high quality research in the form of reference strains, reagents for quality control
- suitable for amplification by PCR
- act as biological control agents

(Sharma and Shouche, 2014)



Bacterial culture collection

- 32 reference bacterial strains
- ATCC and reputable institute
- Cryobank beads, freeze-dried, glycerol stocks
- Maintenance: Working and stock cultures
- Daily cultures
- Viability and purity
- Storage: - 80°C
- Restricted access



Cryobank beads

Preservation of bacterial strains

Freeze dried



Identification of bacterial strains



Genetic Analyzer

Biolog System



Biolog System

Purification of cells



Turbidity of cells

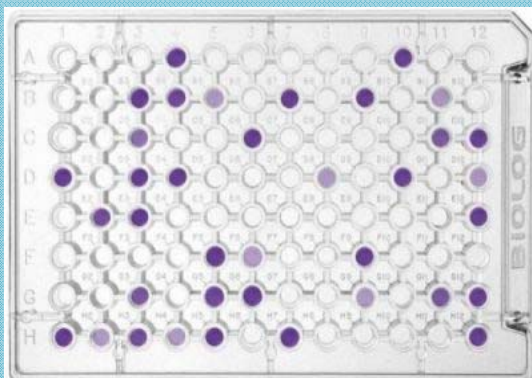


Biolog System




- ❑ Transfer 100µl of inoculation fluid into GEN III microplate
- ❑ Set OMNILOG incubator at 33°C
- ❑ Incubate microplates in incubator for 36 hours

Biolog System



- ❑ Results interpreted automatically based on the utilization of chemicals and substrates in the 96 wells
- ❑ Tetrazolium salt (purple) is used as an indicator to indicate the positive wells of the reactions
- ❑ Bacteria can be identified up to species level.



Challenges



- Sample preparation**
 - sample matrix
 - homogeneity
- Bacterial Cultures**
 - viability
- Contamination**
 - Airborne
 - Reagents
 - Cross-contamination between high and low activity samples

PT outliers in microbiology

- sample handling errors
- inadequate staff training
- lack of understanding of legislation/ guidelines
- inadequate methods
- equipment and culture media failures
- calculation errors
- reporting errors

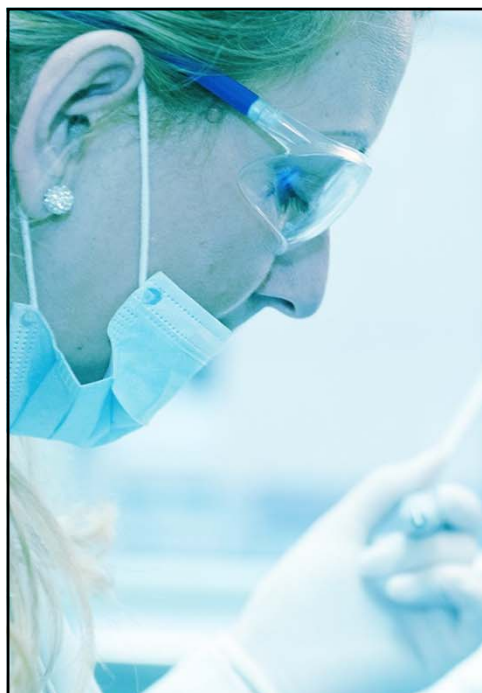


Workflow in microbiology analysis



Training





Future directions

- ❑ To develop more precise low colony-forming unit (CFU) materials for presence/absence tests
- ❑ Development of more trial schemes for both food and water microbiology

References

- ❑ An approach to integrated data assessment in a proficiency test on the enumeration of *Escherichia coli*. Bremser et al., 2010. Journal of Applied Microbiology 110, 128 – 138.
- ❑ Homogeneity and stability of samples used for proficiency testing in enumeration of coagulase positive staphylococci. Jacek et al., 2012. Bulletin Vet Pulaway 56, 559-562.
- ❑ Preparation of reference material for proficiency test for enumeration of coliform in cheese matrix. Brandao *et al.*, 2013. Detection; 1, 7-12.
- ❑ Microbial Culture Collection (MCC) and International Depository Authority (IDA) at National Centre for Cell Science, Pune. Indian. Sharma and Shouche, 2014. J Microbiol. 2014 Jun; 54(2):129–133.

Acknowledgement

