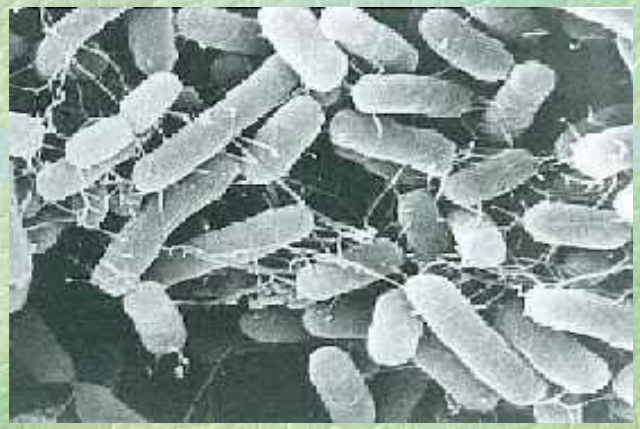
Method Comparisons Between EPA-Method 1682, IMS and PCR for Isolating and Detecting *Salmonella* species from Various Biosolids



Eyob Mazengia

Environmental Lab, Microbiology Unit

Presentation Outline

- Why test for *Salmonella*?
- Objectives
- Relevance to us
- Brief description of methods compared
- Study design
- Results
- Conclusions

Why Test for *Salmonella*?

- Salmonella is one of pathogens of concern in domestic sewage and sewage sludge

- Regulation 503 states:
  - Class A sewage should contain less than 3MPN/4 g of dry total solid

Objectives

- Method comparison between conventional (EPA-1682) and new (IMS, PCR)
- Adaptability to screen for multiple pathogens at the same time
- Turn around time

Relevance to Us

- We currently use a conventional method

- Conventional methods rely on:
  - growth in biological medium to selectively enrich target organisms
  - physical conditions (i.e., antibiotics, pH, temperature) to inhibit non-target organisms

Conventional Method

- **Day 1**:
  - Sample enrichment
- **Day 2**:
  - Transfer from enrichment onto Selective Enrichment containing novobiocin and malachite green which inhibit non-*Salmonella* species

Conventional Method (cont.)

- **Day 3**:
  - Presumptive colonies transferred onto selective and differential medium
- **Day 4**:
  - Confirm colonies with biochemical schemes such as:
    - TSI
    - LIA
    - Urease
- **Day 5**:
  - Serology typing using polyvalent O antisera

Shortcomings

- Underestimates number of target organisms due to sublethal environmental injury
- Very low sensitivity
- Inability of target bacteria to take up nutrient components in the medium
- Inhibitory chemicals/antibiotics used to suppress non-target organisms also reduce the recovery of target organisms

Shortcomings (cont.)

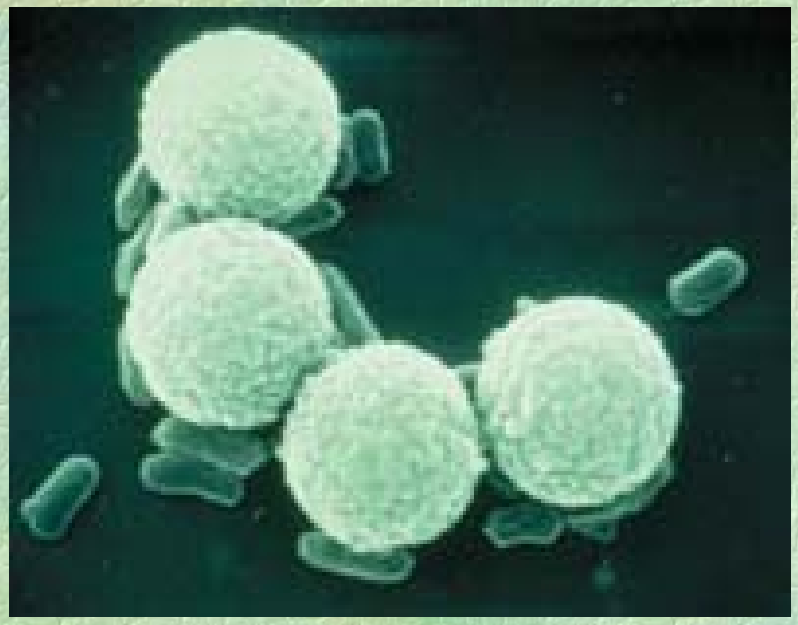
- Time consuming (i.e., 1 to 3 weeks)
- High false negative rate ranging between 22.5 - 35%
- Unable to screen for multiple target organisms at the same time

Immuno-Magnetic Separation (IMS)

- IMS is an antibody based method used for the isolation of viable organisms
- Magnetic beads are coated with *Salmonella* antibodies attract and bind to *Salmonella* growing in broth
- Like the conventional method, IMS relies partly on the use of biological medium both to enrich and detect/recover organisms

IMS Procedure

- **Day 1:**
  - Samples are enriched (i.e., TSB)
- **Day 2:**
  - Add magnetic beads coated with anti-*Salmonella* surface antibody
    - Harvest and wash magnetic beads
    - Enrich bacteria-bead complex

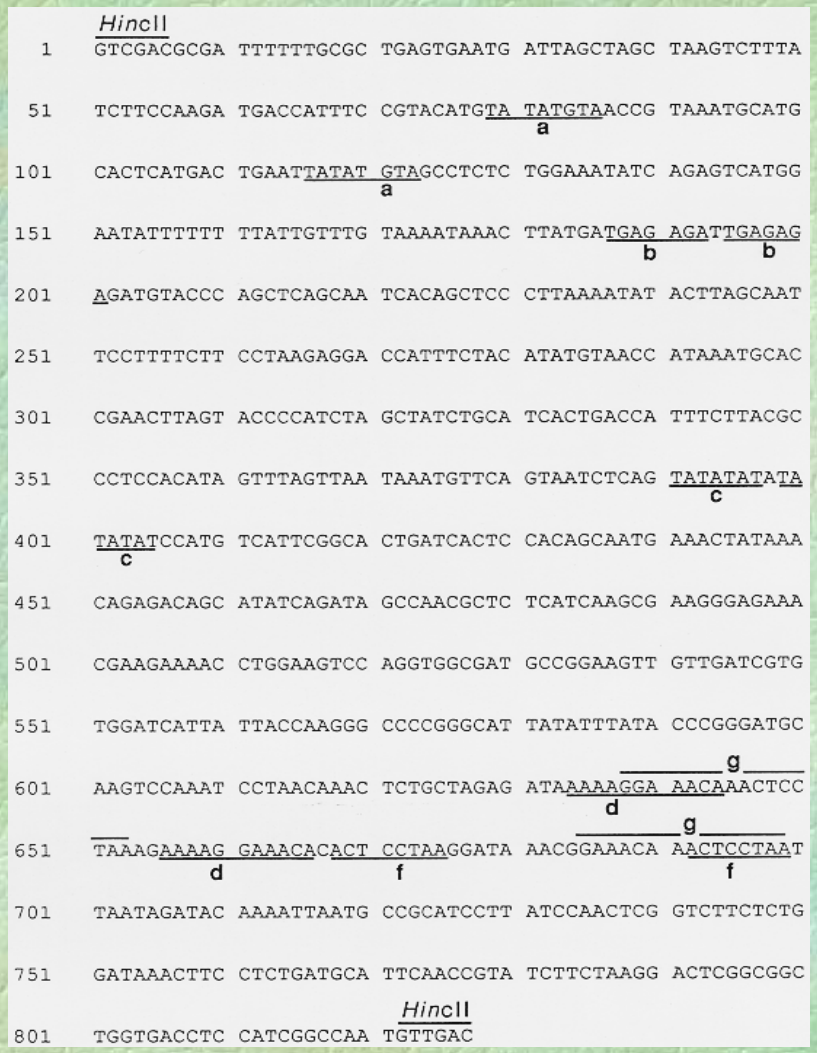


IMS Procedure (cont.)

- **Day 3:**
  - Streak the enriched bacteria-bead complex onto selective medium appropriate for *Salmonella*
- **Day 4:**
  - Pick presumptive *Salmonella* colonies and transfer into enrichment
- **Day 5 & 6:**
  - Confirm with serology or biochemical ID scheme

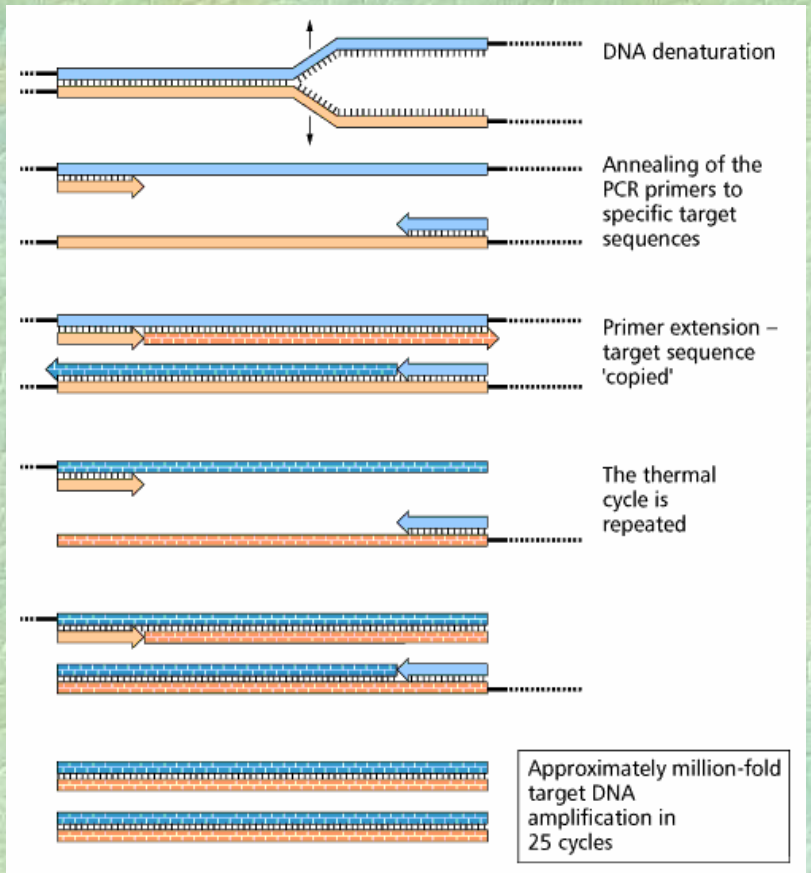
Polymerase Chain Reaction (PCR)

- PCR is a gene based method targeting unique DNA sequences only found in *Salmonella*
- Once a signature sequence is identified, it is used as a primer



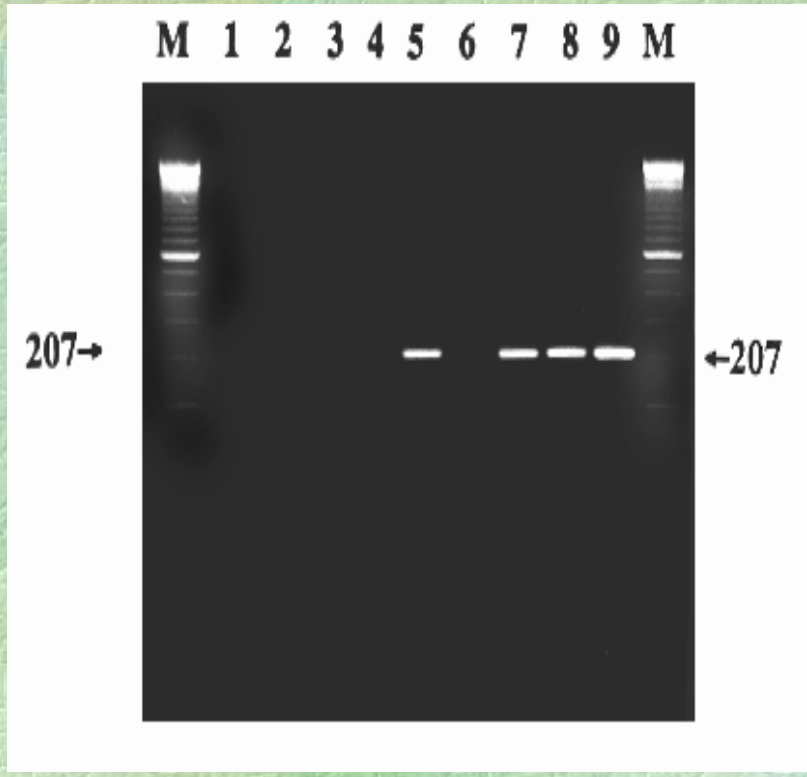
PCR Procedure

- **Day 1:**
  - Enrichment
- **Day 2:**
  - Cell Lysis
  - PCR amplification
  - Gel electrophoresis
  - Analysis



PCR Procedure (cont.)

- The presence or absence or genes complementary to the primer used are detected using agarose gel electrophoresis



Study Design

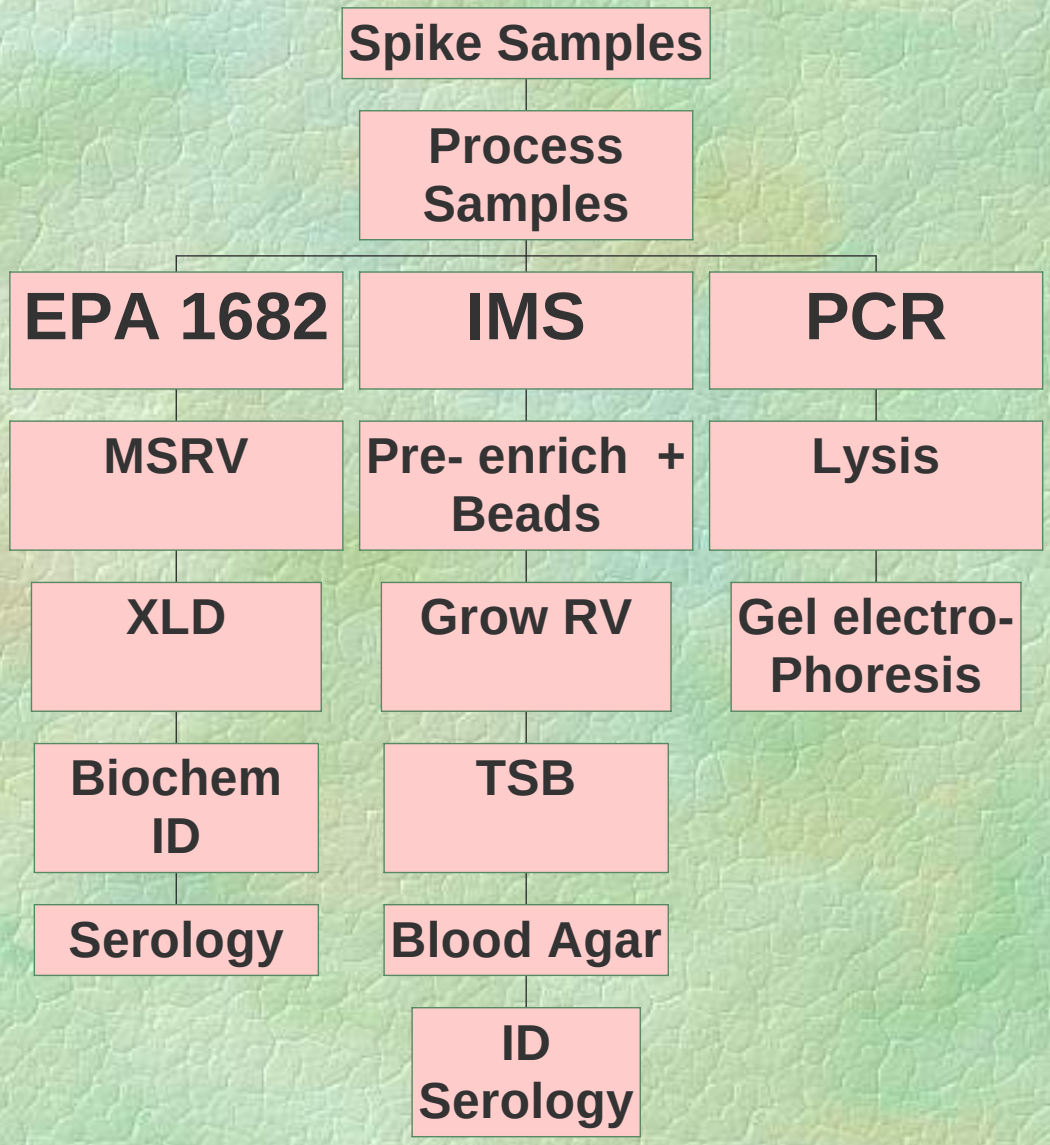
- A total of 16 samples and four different types of biosolids were included in the study
  - Compost
  - Thermophically digested (liquid),
  - Alkaline stabilized (Solid)
  - Milorganite (heat-dried solid)

- The Most Probable Number (MPN) method was used to quantify the number of *Salmonella* present in the sample

Study Design (cont.)

- There were 360 individual tests per method (20/sample)
- Samples were all spiked with either bioball or laboratory prepared spikes and enriched in TSB and processed according to each protocol

Study Design



Results

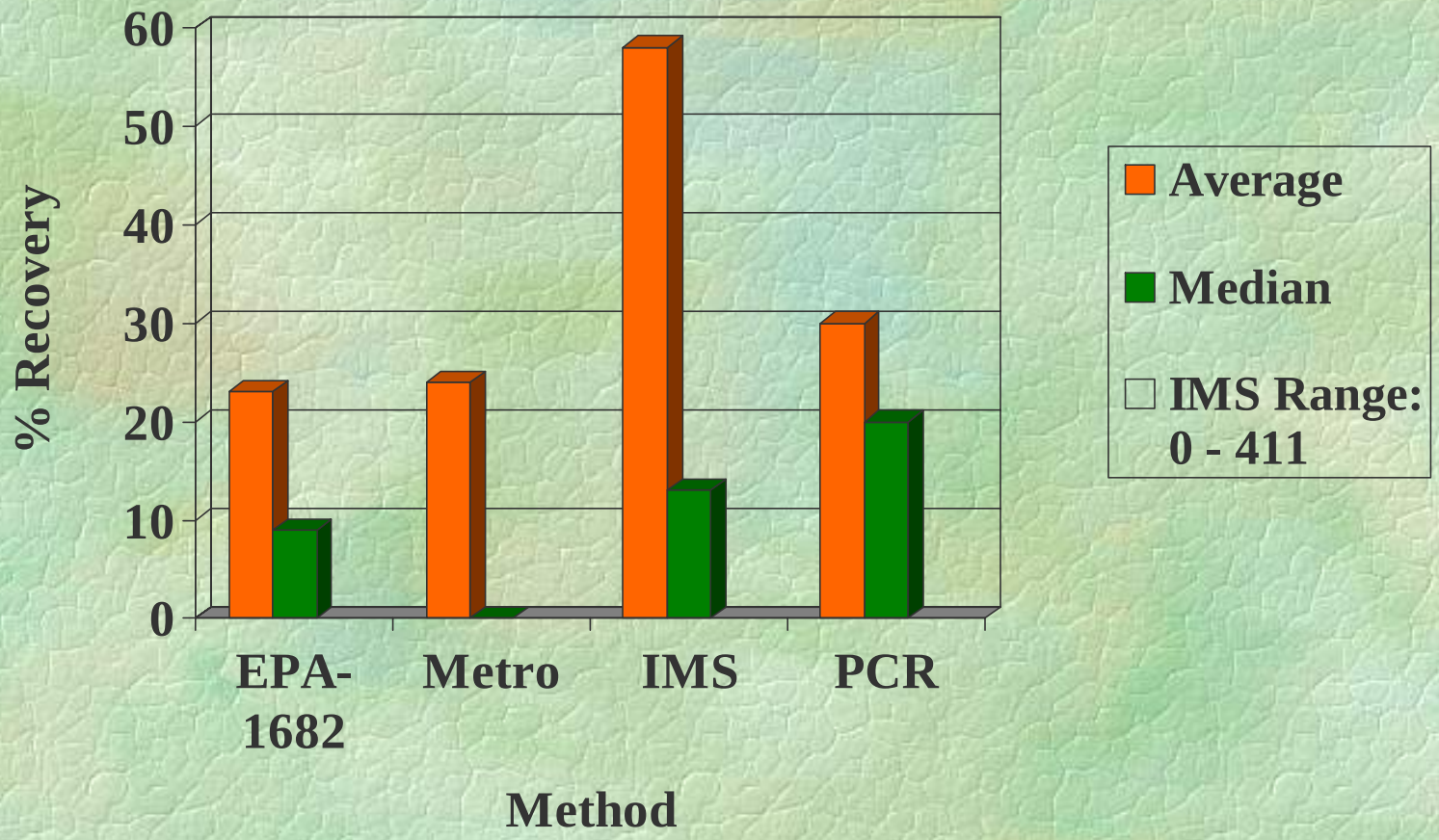
- Average % recovery of the methods (all matrix combined)
- Average % recovery of the methods by matrix
- Matrix interference
- Average number of days to complete the test
- Adaptability to screen multiple pathogens

Average Nominal % *Salmonella* Recovery

(Measured against known inocula)

% Recovery	EPA-1682	Metro-Mod-EPA	IMS	PCR
Average	23	24	58	30
Ranges	0-79	0-91	0-411	0-98
Median	9	0	13	20

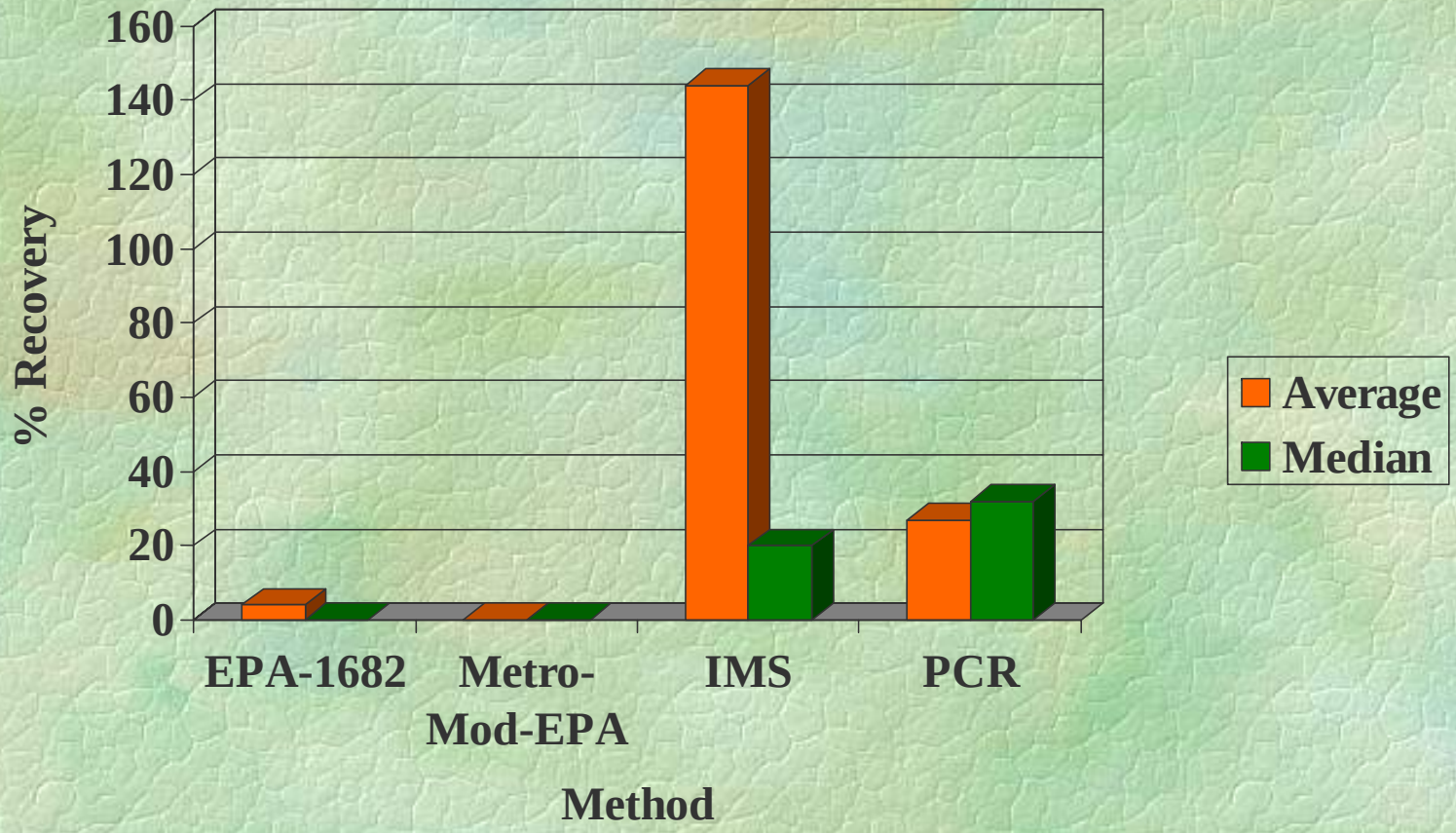
Average Nominal % *Salmonella* Recovery



% Nominal Recovery of *Salmonella* from Compost (3 samples/Matrix).

% Recovery	EPA-1682	Metro-Mod-EPA	IMS	PCR
Average	4	0	144	27
Ranges	0-13	0	1-411	7-43
Median	0	0	20	32

% Nominal Recovery of *Salmonella* from Compost



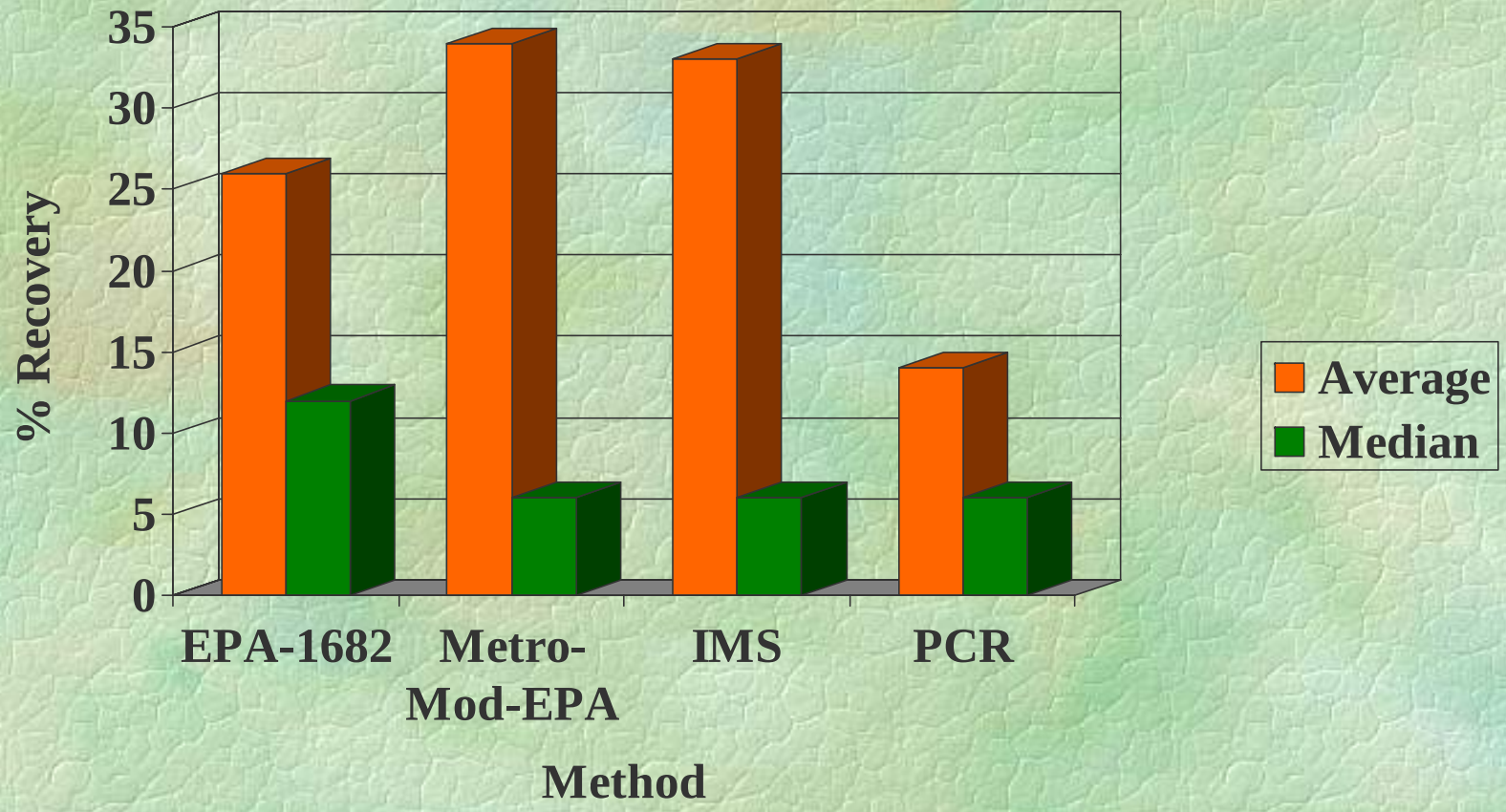
% Nominal Recovery of *Salmonella* from Thermophilically Digested Sludge (liquid)

% Recovery	EPA-1682	Metro-Mod-EPA	IMS	PCR
Average	0	0	0	0
Ranges	0	0	0	0
Median	0	0	0	0

% Nominal Recovery of *Salmonella* from Alkaline-Stablized (3 samples/Matrix).

% Recovery	EPA-1682	Metro-Mod-EPA	IMS	PCR
Average	26	34	33	14
Ranges	6-61	6-91	6-93	0-35
Median	12	6	6	6

% Nominal Recovery of *Salmonella* from Alkaline-Stabilized

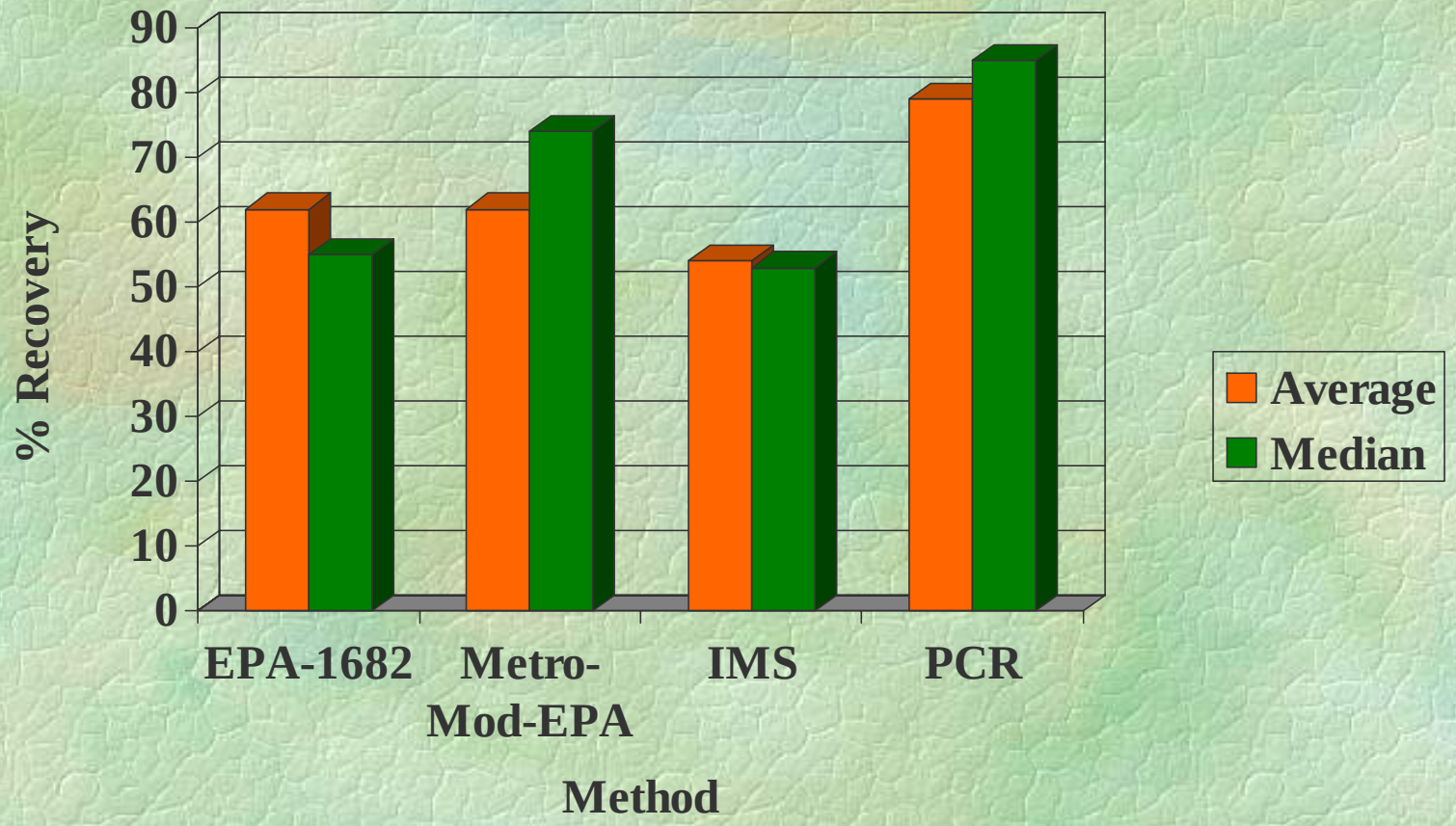


% Nominal Recovery of *Salmonella* from Milorganite (3 samples/Matrix).

(Recovered *Salmonella* in unspiked sample)

% Recovery	EPA-1682	Metro-Mod-EPA	IMS	PCR
Average	62	62	54	79
Ranges	52-79	34-79	36-74	54-98
Median	55	74	53	85

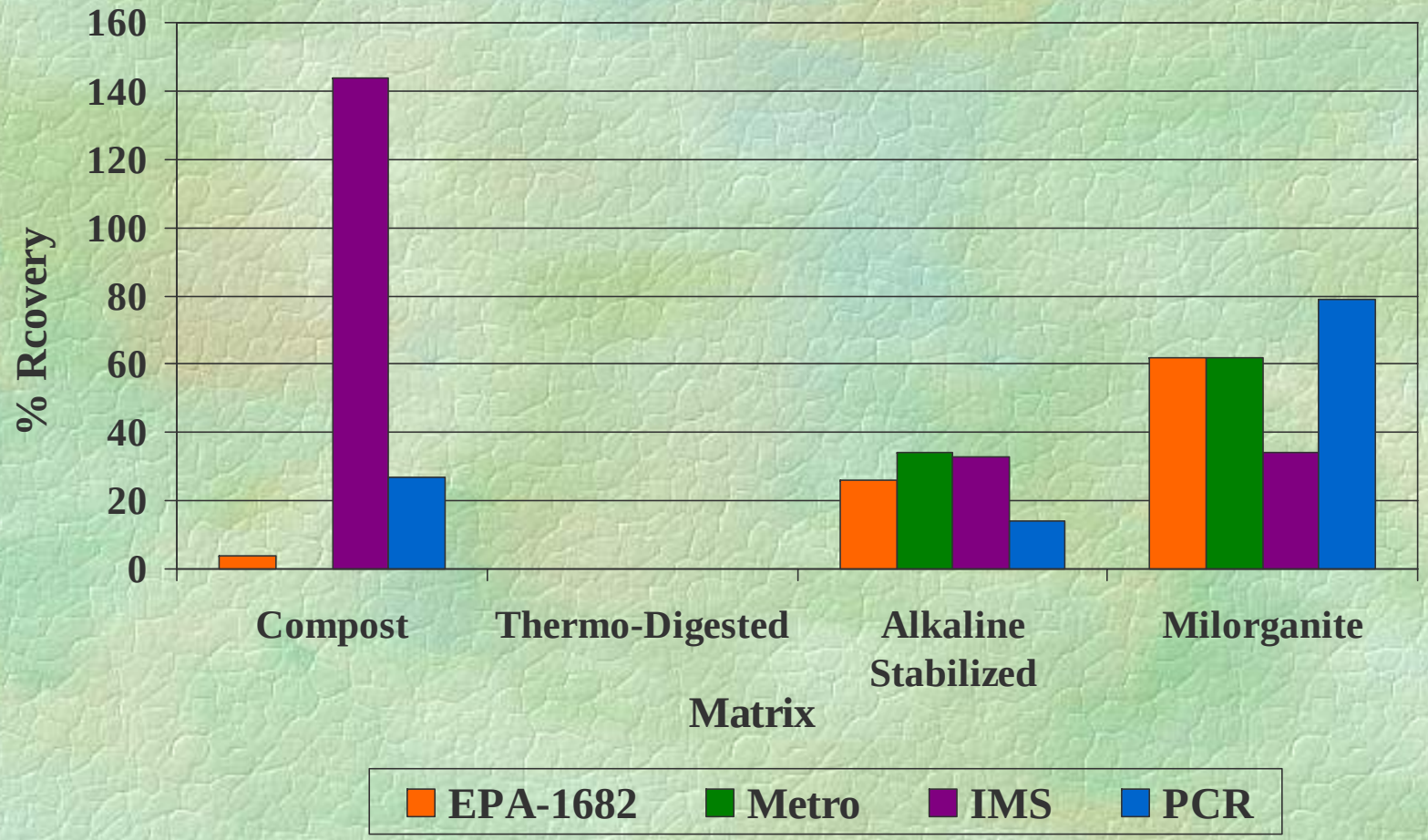
% Nominal Recovery of *Salmonella* from Milorganite



Summary of % Nominal Recovery of *Salmonella* by Matrix (3 samples/Matrix) *411% recovery

Matrix	**EPA-1682**	**Metro-Mod-EPA**	**IMS**	**PCR**
Compost	4	0	144 *	27
Thermop-digested	0	0	0	0
Alkaline stablized	26	34	33	14
Milorganite	62	62	54	79

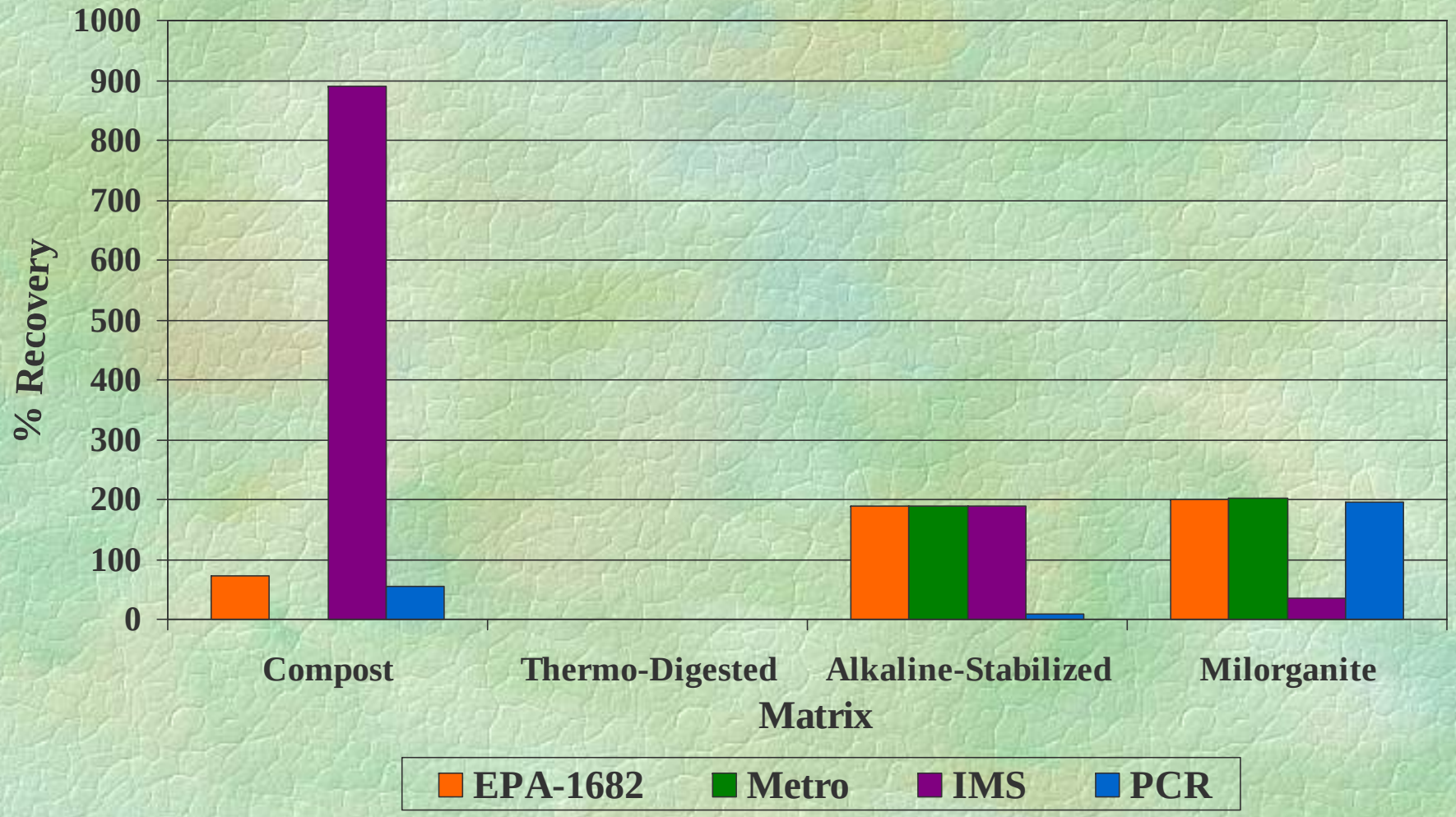
Summary of % Nominal Recovery of *Salmonella* by Matrix



Summary of % Recovery Using Alternate Log Phase Lab Spike (1 sample/matrix)

Matrix	**EPA-1682**	**Metro-Mod-EPA**	**IMS**	**PCR**
Compost	72	0	891	55
Thermop-digested	0	0	0	0
Alkaline stablized	189	189	189	9
Milorganite	201	202	35	196

Summary of % Recovery Using Alternate Log Phase Lab Spike



Other Method Limitations

	EPA-1682	Metro-Mod-EPA	IMS	PCR
Average days	6	5	6	2
Screen multiple pathogens	No	No	Yes (limited)	Yes

Conclusions

- Matrix interference is a major concern therefore different methods should be used for different types of biosolids
- IMS method is similar to the culture method but it provides a clean-up step to remove inhibitory agents, chemicals and competing background bacteria. This appears to enhance recovery
- Alkaline stabilized samples can initially be enriched by IMS and detected with PCR

Conclusions (cont.)

- Combining the two approaches (IMS and PCR) may save us time in all matrices and increase sensitivity in detecting and isolating *Salmonella* species in Compost and Milorganite biosolids
- PCR's reduction in processing time enables a larger number of samples analyzed in the same time period
- Because both IMS and PCR methods are new, they have not yet been recognized for regulatory work, but they can still be used for non regulatory work