# Detection Methodology

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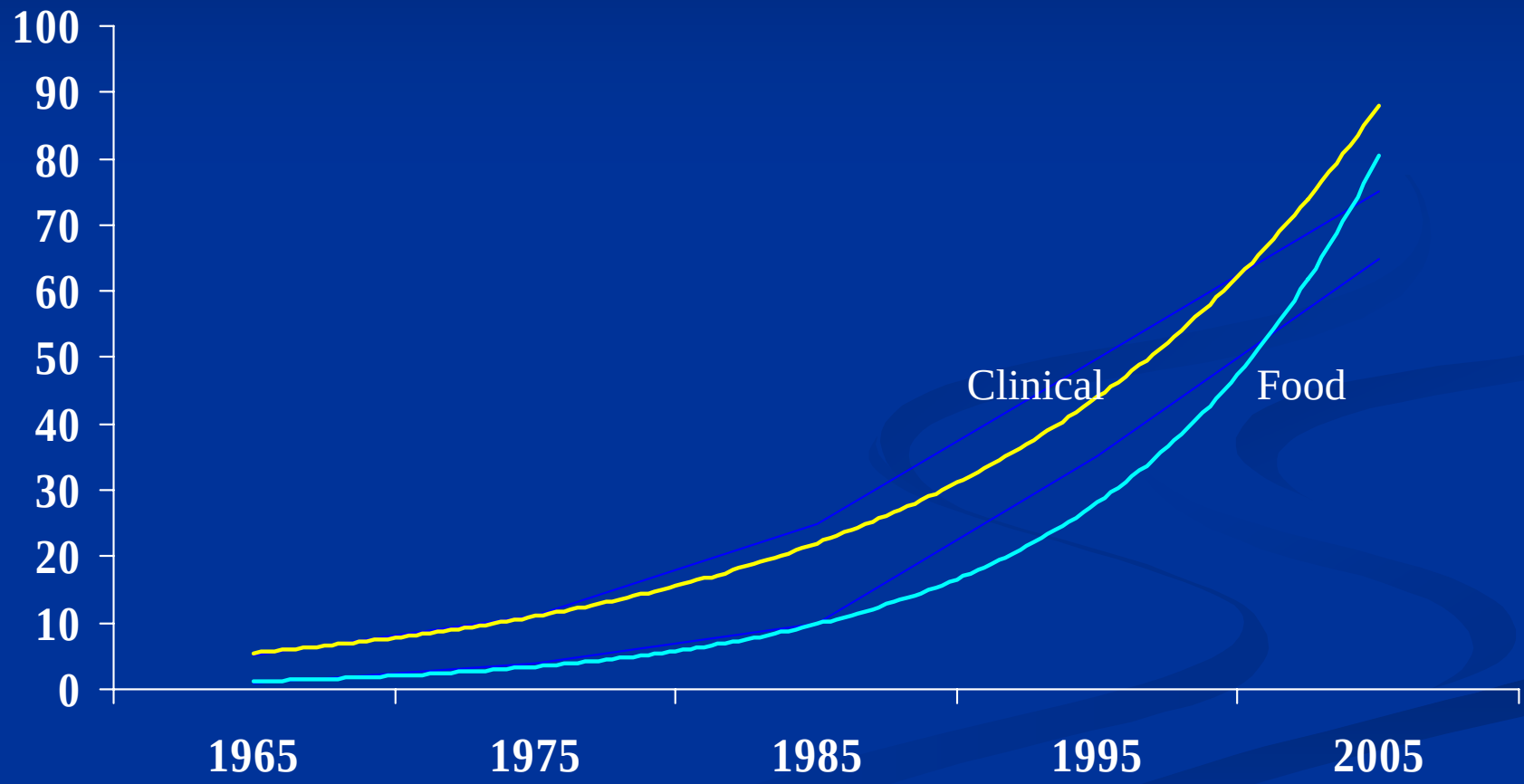
# Evolution of rapid methods for pathogen detection



# What is an ideal pathogen test?

- Real time results
- Fully automated
- Little training needed to run
- Inexpensive
- Results automatically linked into data management system
- No false positive or false negative results

# Cultural Methods
# - Time Line -

- Preenrichment – 24 hr
- Enrichment – 24 hr
- Plating – 24 hr
- Screening – 24 hr
- Serology – 1 hr

- Total – 4 days

# Cultural Methods

- Preenrichment (35-37 C) for 18 to 24 hr
  - FSIS and most use Buffered Peptone water (BP)
  - FDA and some use lactose broth (Universal Preenrichment for fruit juices)
  - For dry products Universal Preenrichment works best
  - Some rapid methods use 6 to 8 hr preenrichment before overnight enrichment
  - Some highly contaminated wet products sometimes do not use preenrichment (direct enrichment)

# Cultural Methods

- Enrichment (35 – 37 or 42 C) for 18 to 24 hr
  - FSIS, FDA and most use TT at 37 and RV at 42
  - Most no longer use selenite cystine because of disposal problems
  - For fecal samples, NARMS uses first stage enrichment in GN (24 hr at 37 C) and Tet (48 hr at 37 C). Transfer to RV and enrich for 24 hr at 37 C.

# Cultural Methods

- Selective Plating Media (24 hr at 37 C)
  - FSIS: MLIA, BG sulfa, XLT4
  - FDA: Hektoen Enteric, XLD, Bismuth Sulfite
  - NARMS: XLT4, BG sulfa
- Screening Media (24 hr at 37 C)
  - TSI and LIA
- Grouping and Confirmation
  - Somatic ('O') and Flagellar ('H') antisera

# "Rapid" Methods

- ELISA – 48 hr
  - Tecra, Vidas, BioControl
- Enhanced (concentrated) ELISA – 24-28 hr
  - Tecra, BioControl
- DNA probe – 48 hr
  - GeneTrak
- Commercial PCR – 24 – 28 hr
  - Bax
- Antibody precipitation – 24 – 48 hr
  - 1-2 test, multiple company Lateral Flow devices

# FSIS methodology

- Screening method:  BAX
- Confirmation method:  Cultural
  - BP
  - TT, RV
  - MLIA, BG sulfa
  - TSI, LIA
  - serology

# What is the cost of pathogen testing?

- Highly variable depending on fixed costs!
- Conventional - $1 to $4
- most 48 hr assays - $2.50 to $5.00
- most 24 hr assays - $3.50 to $10.00
- biosensors etc. - unknown but probably higher

# Considerations for Adoption of Methods

- Precision
  - Sensitivity and Specificity
  - Quantitative equivalence
  - Repeatability
- Ease of use
- Number and types of samples
- Cost
- AOAC approved

# Essential Considerations

- Sampling plan and sample handling on the front end of procedure are critical
- Physiological condition of *Salmonella* plays a role in determining optimal media and methods
- Media bias – growth factors, buffering, chemicals, and antibiotics in media favor certain populations and discourage others
- Automation

# Required sample size for 95% certainty of detection with a sensitivity of 100%

| Flock size | Prevalence (%) | | | | |
|---|---|---|---|---|---|
| **No. birds** | **50** | **25** | **10** | **1** | **0.1** |
| 20 | 4 | 9 | 16 | 20 | 20 |
| 100 | 5 | 10 | 25 | 96 | 100 |
| 1000 | 5 | 11 | 29 | 258 | 950 |
| 5000 | 5 | 11 | 29 | 290 | 2253 |
| 10,000 | 5 | 11 | 29 | 294 | 2995 |

# Quantitation
# - New procedures needed -

- MPN procedures cumbersome and expensive
- Risk Assessments require enumeration, not just presence/absence
- Direct plating and real time PCR are problematic because most poultry and meat *Salmonella* levels are less than 100 viable cells/carcass or unit area and the sensitivity of direct plating is poor unless there are a minimum of 500 to a 1000 cells per carcass or unit area

# For Example

- 80 to 95% of *Salmonella* positive chicken carcasses have less than 100 cells – total. No more than 50 of these cells will be removed in a 100 mL rinse. Under ideal conditions (which never exist) you would only have 1 cell for every 2 mL of rinse. A direct plate procedure which uses 0.5 mL (maximum that can be spread on a very dry plate) would have a high probability of no detectible cells (colonies). When you combine backgrounds of fat, protein and high numbers of competitive organisms then the problem is apparent

# What to do?

- Some possibilities:
  - Concentration: Using either centrifucation or filtration it is possible to get as much as a 100 fold increase in concentration (sensitivity)
  - Highly selective media to prevent competitive bacteria from overgrowing or mimicking *Salmonella*, but must guard against sensitivity of the *Salmonella* to the selective pressures of the chemicals or antibiotics.
  - Develop automated MPN procedure. Sensitive and accurate procedure likely to be very expensive.

# Tracking or Discrimination Methodologies

- discriminatory
  - must distinguish between truly distinct clones
  - must not be too discriminatory so as to call all isolates individual clones
  - must not be too dependent upon genetic drift (random mutation)

# Requirements for Tracking Methodologies

- determine genetic distances between different but related strains
  - provides information as to the spread and stability of the population
- technically simple
- rapid/high throughput
- readily applied to a different organism
  - initial setup equipment and reagents should be transferable to the analysis of a different organism

# Requirements for Tracking Methodologies

- reproducible
  - intralaboratory
  - interlaboratory
- cost effective
  - per sample
  - applied to a different organism

## phenotypic methods - serotype, biotype, phagetype

- ☑ discriminatory

  determine genetic distances between different but related strains

  technically simple

  rapid/high throughput

  readily applied to a different organism

- ☑ reproducible

  cost effective

# Common Methods Employed in Characterization & Epidemiological Tracking of Pathogens

**genotypic methods**

- RAPD {**R**andomly **A**mplified **P**olymorphic **D**NA}
- PCR-RFLP {*flaA* **R**estriction **F**ragment **L**ength **P**olymorphism}
- ribotype
- AFLP {**A**mplified **F**ragment **L**ength **P**olymorphism}
- PFGE {**P**ulse **F**ield **G**el **E**lectrophoresis}
- direct nucleotide sequencing

# Key to success of tracking or discrimination technologies

- The value of PulseNet is to a certain extent in the technical ability of PFGE to distinguish clonal bacteria, but more importantly is that it allowed comparison of patterns from multiple and discrete laboratory locations.
- More discriminatory technology must be able to be run consistently by different laboratories and the data base must be robust and accessible to those laboratories so that comparisons can be readily determined.

# Stan's crystal ball
## -- cutting edge technologies --

- Today - PCR, immunoconcentration
- 3 to 5 years - multiplexed PCR, 8 to 16 hour automated detection
- 5 to 10 years - biosensors, microarray chip technology with 0 to 4 hour pathogen detection