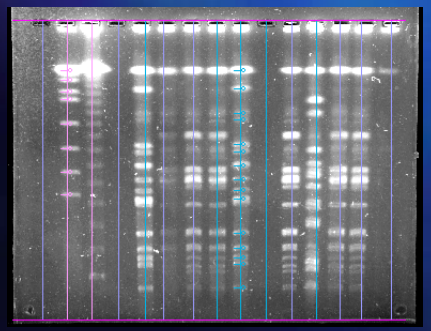
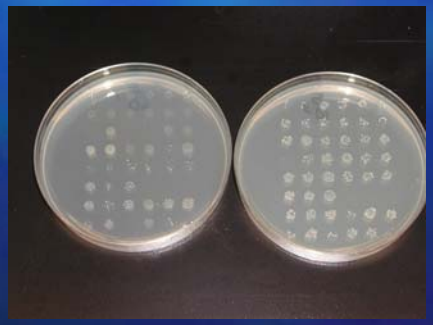
# Bacterial Source Tracking Methodologies

- Salisbury University
  - Mark F. Frana
  - Elichia A. Venso
- Maryland Department of the Environment
  - Lee Currey
  - Kathy Brohawn





# Bacterial Source Tracking (BST)

- Determination of sources of fecal bacteria from environmental water samples (1995)
- Several different techniques are currently being used for BST
- Many current techniques are “Library-based”

# BST Technology

- Genotypic
  - Pulsed-Field Gel Electrophoresis (Simmons/Herbein/Hagedorn – Va. Tech.)
  - Ribotyping (Samadpour – U. of Wash.)
  - Randomly Amplified Polymorphic DNA (RAPD)/PCR (Sadowsky – U. of Minn.)

# BST Technology

- Biochemical (Phenotypic)
  - Multiple Antibiotic Resistance (MAR) (Hagedorn - Va. Tech/ Kator – VIMS/ Wiggins – James Madison)
  - Coliphage (Geoff Scott, Jill Stewart – NOAA)
  - Carbon Source Profiles (Hagedorn – Va. Tech)
- Chemical (Non bacterial)
  - Optical Brighteners
  - Caffeine Detection

# Fecal Indicators (Microbial)

- Fecal Coliforms (*E. coli* )
  - Facultative, Gram negative, non-spore forming, rod – shaped, ferment lactose producing gas within 48 hours. Sources: animals, soil (44.5$^0$ C is selective)
- Fecal Streptococci (Enterococci)
  - Facultative, Gram positive, tolerate high salt and are used as indicators particularly in brackish or marine waters. Sources: warm-blooded animals

# Project Overview

- Monitor study sites for elevated levels of fecal indicator
- Isolate indicator organism from known sources (for “library”)
- Isolate indicator organism from water samples
- Analyze all isolated organisms using BST method
- Predict probable sources of fecal indicator in the water by statistically comparing water and library isolates

# MAR Analysis

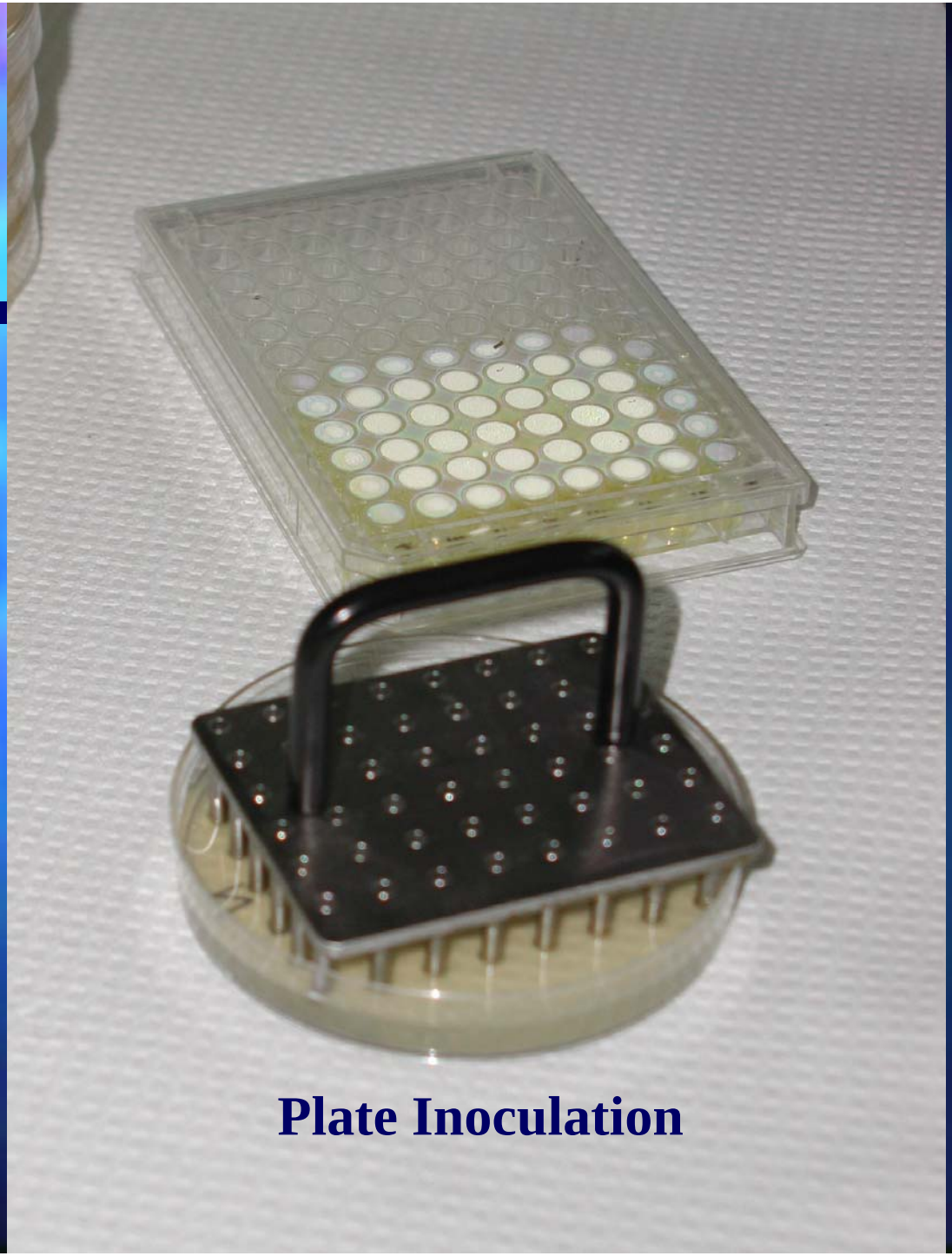
- Unknown Sources (water)
  - Membrane filtration
  - Colony enumeration
  - Inoculation of unknown sources onto antibiotic plates
- Known sources
  - Inoculation of known sources onto antibiotic plates
- Read and record resistance profiles
- Data entry and statistical analysis

# Multiple Antibiotic Resistance

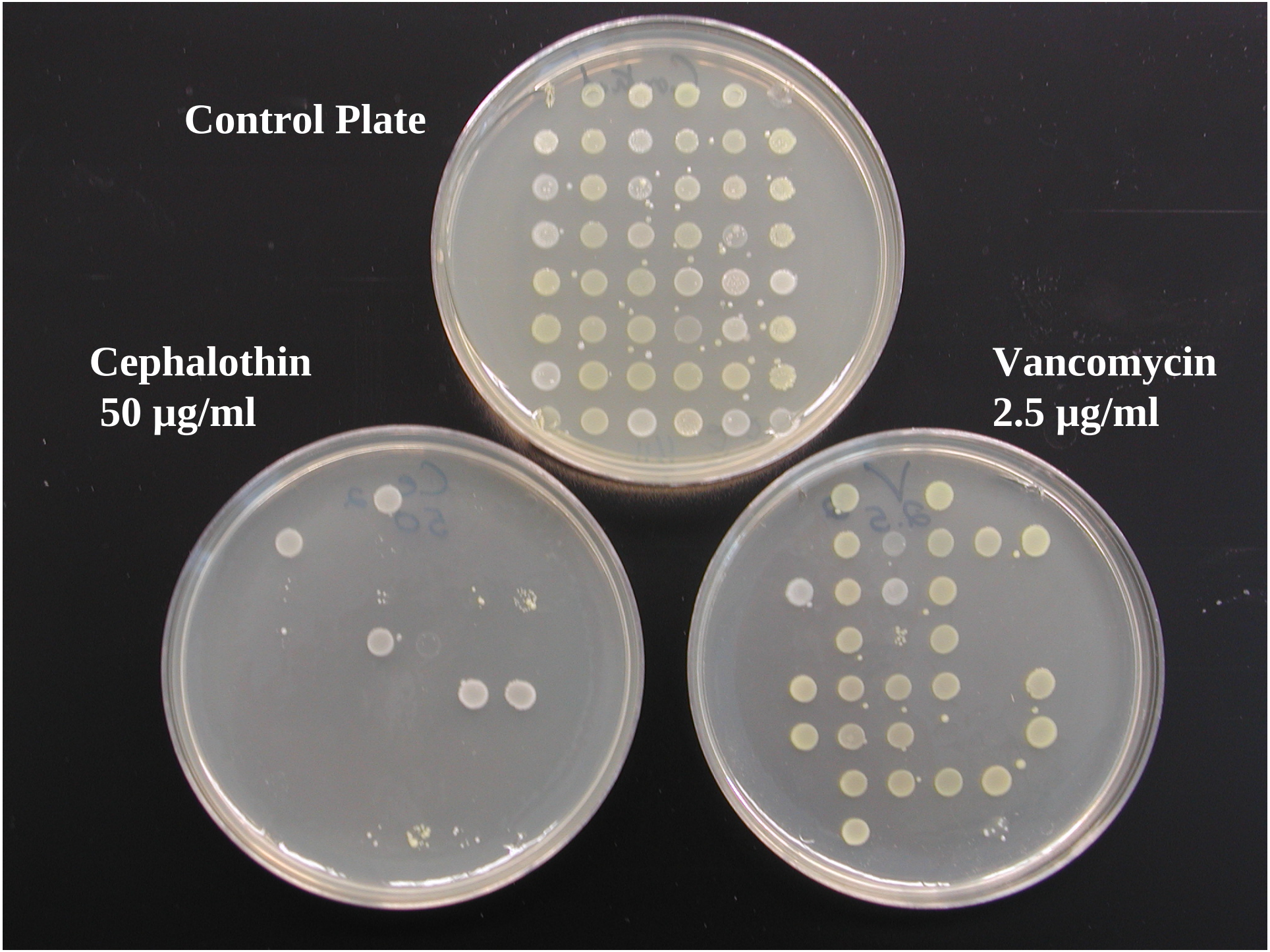
- 35 combinations of concentrations using 12 different antibiotics
- Inoculate each antibiotic containing plate with 48 isolates
- Incubate for 24 h at 35 $^{0}$C
- Read plates
  - 1=isolate grew (resistant)
  - 0=isolate did not grow (susceptible)

# Antibiotics & Concentrations (µg/ml) Used in Analysis

- Amoxicillin: 0.625
- Cephalothin: 10, 15, 30, 50
- Chloramphenicol: 2.5, 5, 10
- Chlortetracycline: 60, 80, 100
- Erythromycin: 10
- Gentamycin: 5, 10, 15
- Neomycin: 40, 60, 80
- Oxytetracycline: 20, 40, 60, 80, 100
- Salinomycin: 1, 2.5, 10
- Streptomycin: 40, 60, 80, 100
- Tetracycline: 10, 30, 50, 100
- Vancomycin: 2.5

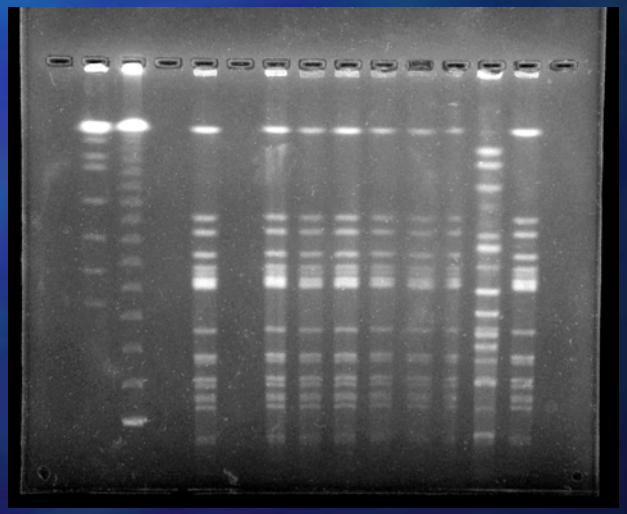


# Plate Inoculation


Control Plate
Cephalothin
50 µg/ml
Vancomycin
2.5 µg/ml

# Pulsed-Field Gel Electrophoresis

- Fragmented DNA from each bacterial isolate separated by electric current
- Banding patterns of library isolates are compared to water isolates using computer software



# Library-Building

- Collection of known-source scat
- Isolation of individual bacterium from scat (isolate)
- Response of isolates to 35 antibiotics concentrations → library

# Probable Source Categories

Categories based on potential management remediation:

- Pets – public education, outreach
- Human – infrastructure/septic system repair and/or replacement
- Livestock – adherence to BMPs
- Wildlife – no management intended

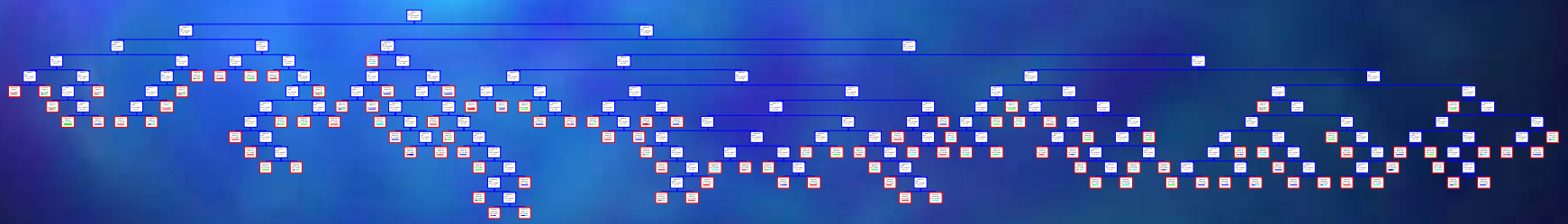
# Statistical Methods in MAR

- Discriminant analysis – most often used
- Logistic regression
- Classification Tree Software - SU
  - Developed by Stanford University
  - CART®
    - Predictive
    - Uses all of data to maximize predictive ability
  - Recursive Binary Partitioning
    - Partition at each step based on the variable (antibiotic) that maximizes correct classification

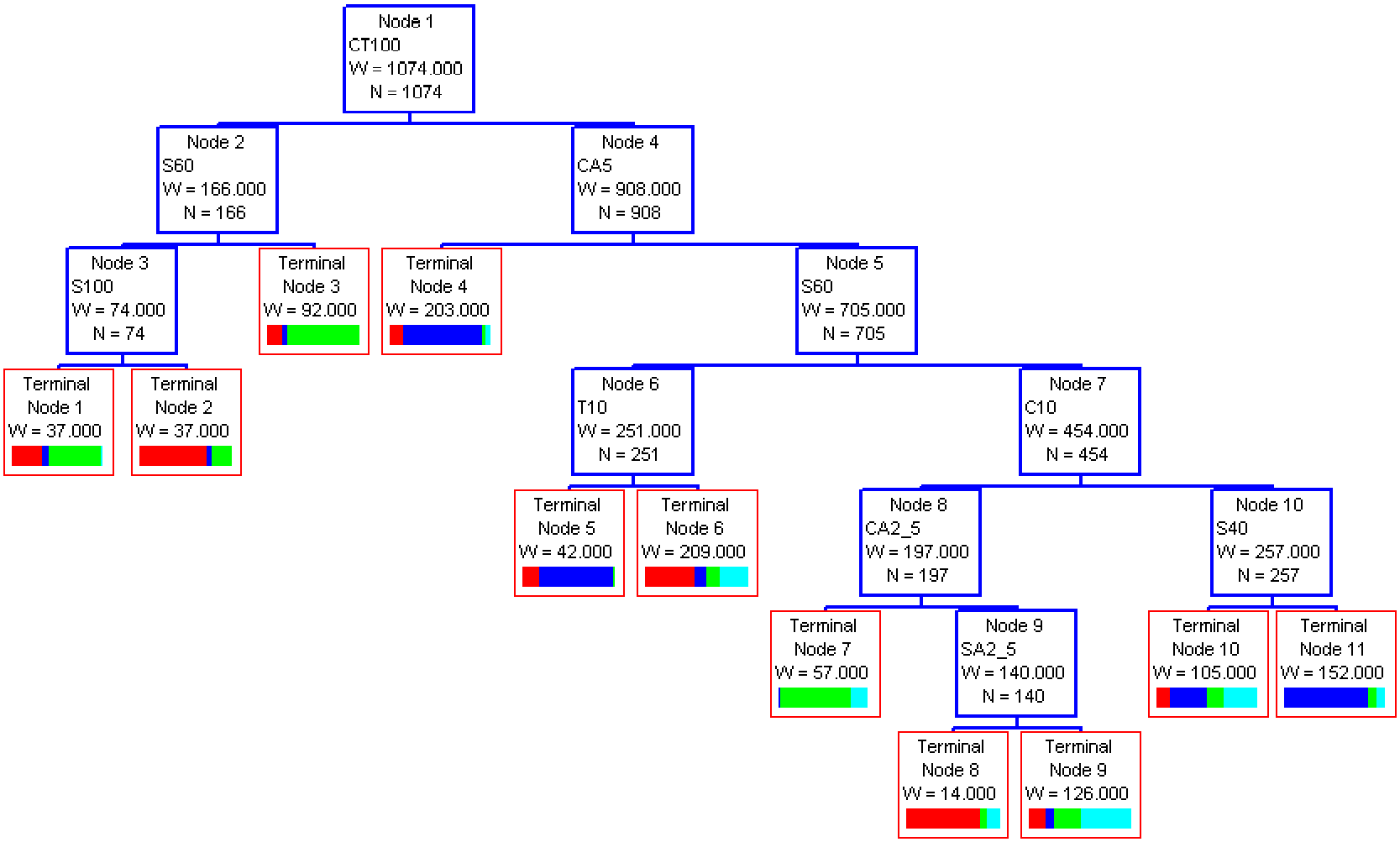
# Classification Tree

Terminal Nodes define the classification Model

Schematic of Full Model

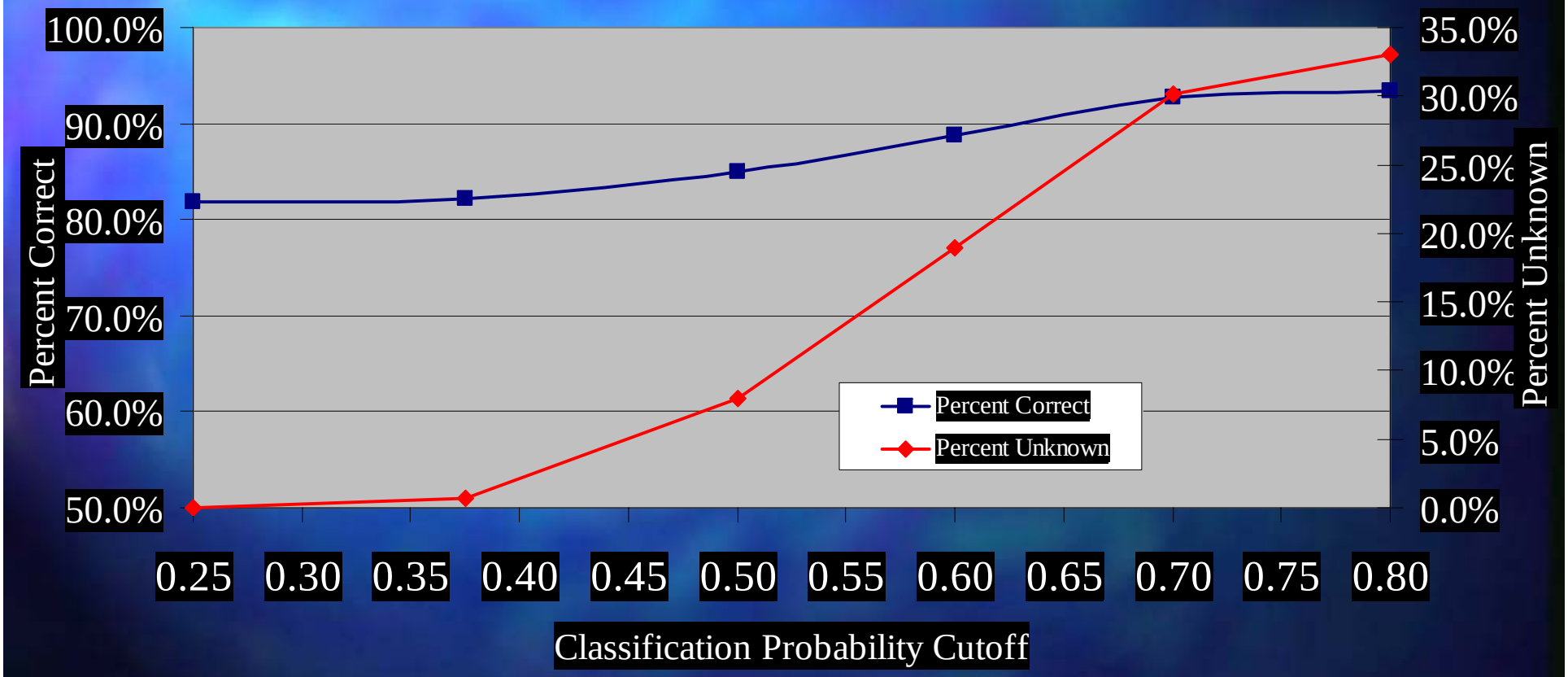


# Partial Model - “Pruned” to 11 Terminal Nodes



# Classification Probability Levels

## Determination of Probability Cutoff



# Model Testing

- Cross-validation
  - Remove 10% of isolates
  - Develop classification model with CART®
  - Run the 10% of the library using model
  - RCCs for the 10% unknowns >90%
- Library treated as unknowns
  - RCCs >90% for pet, human, livestock
  - RCC for wildlife >70%
- Assume water isolates will be classified with same ARCC

# Classification: Library

**Rates of Correct Classification by Category**

| | |
|---|---|
| Pet | 93% |
| Human | 97% |
| Livestock | 93% |
| Wildlife | 71% |

RCC = number of correctly predicted species / Total number predicted.

Example: One hundred sixty-three (163) Pet correctly predicted / 175 total number predicted for Pet = 163/175 = 93%.

# Conclusions:

- Several successful methods exist for BST analysis.
- Each method has known advantages and disadvantages.
- MAR is the current method of choice at Salisbury University for the State of Maryland.
- Statistical analysis of correct rates of classification support the validity of this approach.

# Acknowledgements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