Rapid Methods and Automation in Microbiology: 30 Years of Development and Predictions

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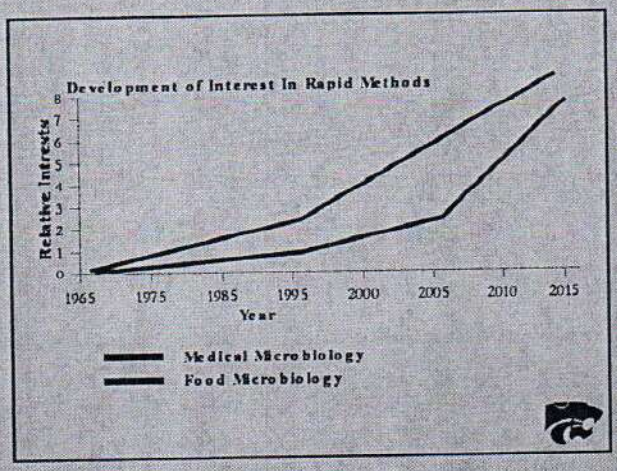


FOOD MICROBIOLOGY

- Sample preparation
- Total viable cell count
- Differential cell count
- Pathogenic organisms
- Enzymes and Toxins
- Metabolites and Biomass

Table 1. New pathogens that are foodborne and pathogens newly recognized as predominantly foodborne in the United States in the last 20 years

Campylobacter jejuni
Campylobacter fetis ssp. *fetus*
Cryptosporidium cayetanensis
Escherichia coli O157:H7 and related *E. coli* (e.g. O11:NM, O104:H21)
Listeria monocytogenes
Norwalk viruses
Nitzchia pungens (cause of amnesic shellfish poisoning)
Salmonella Entertidis
Salmonella Typhimurium DT 104
Vibrio cholerae 01
Vibrio vulnificus
Vibrio parahaemolyticus
Yersinia enterolitica

Methods for the detection of *Escherichia coli* O157:H7 in Foods

1. Conventional Methods-time honored, "Gold Standard", upto5 days
2. ELISA-Enzyme Linked Immunoabsorbant Assay-manual and automated
3. Dipsticks-rapid detection after enrichment
4. DNA/RNA Probes
5. PCR-Polymerase chain reaction and many modifications
6. Ribotyping-pin-point source of contamination
7. Epifluorescence Microscopy
8. Electrochemical reactions
9. Fiber-Optic Biosensor
10. Fluorescent Bacteriophage
11. Light Addressable Potentiometric Sensor
12. Electrochemiluminescent Detection of Immunomagnetic captured antigens

Table 4. Numbers of Total Plate Count, Coliform Count, and specific pathogen tests performed by respondents

Tests	# Doing the test				Average #			
	1981-7 (55)	1989 (31)	1990 (34)	1991 (41)	1981-7 (55)	1989 (31)	1990 (34)	1991 (41)
Total Plate counts	44	25	26	31	12,786 (250,000)*	16,378 (182,500)	25,509 (73,000)	12,585 (60,000)
Coliform counts	43	10	24	31	25,023 (840,000)	4,546 (10,000)	10,112 (73,000)	10,558 (50,000)
Salmonella	34	23	19	23	1,500 (15,000)	3,753 (18,000)	2,278 (10,000)	2,955 (36,000)
Listeria	12	9	12	17	520 (15,000)	2,900 (12,000)	1,740 (8,000)	750 (4,800)
Campylobacter	5	2	5	10	4 (100)	250 (400)	330 (1,500)	187 (500)
S. aureus	6	8	3	6	330 (6,000)	2,370 (7,200)	284 (500)	3,009 (6,000)
E. coli	6	7	2	3	2,000 (50,000)	2,543 (8,000)	5,250 (10,000)	2,100 (5,000)

Numbers in parenthesis indicate number of respondents.

* Maximum number of tests done

One Shift Pathogen Tests – 6 to 8 hr

Validated

Neogen *E. Coli* O157:H7 – 8 hr test

Under development

Umedic *E. Coli* O157:H7 – 8 hr test

Detex *E. Coli* O157:H7 – 8 hr test

MicroStar *E. Coli* – 8 hr test

Real Time Results – Minutes

RBD2100 – viable cell counts – 30 minutes

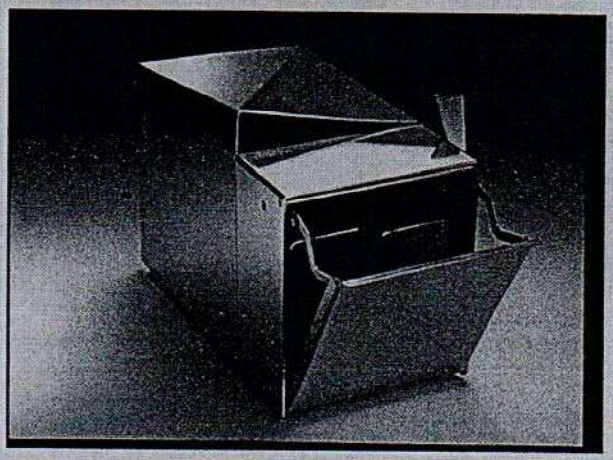
DEFT – viable cell counts – 60 minutes

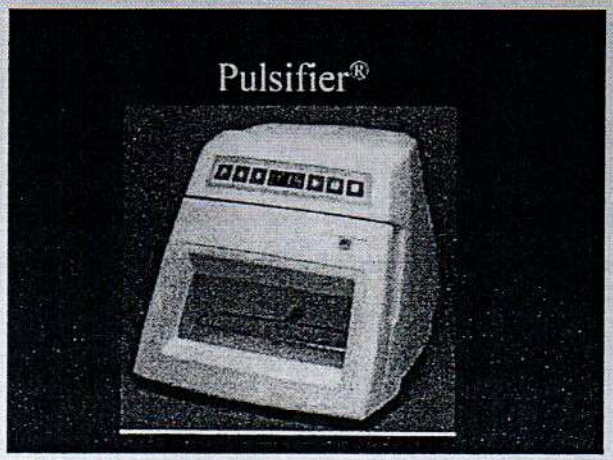
Aflatoxin tests –10 – 20 minutes

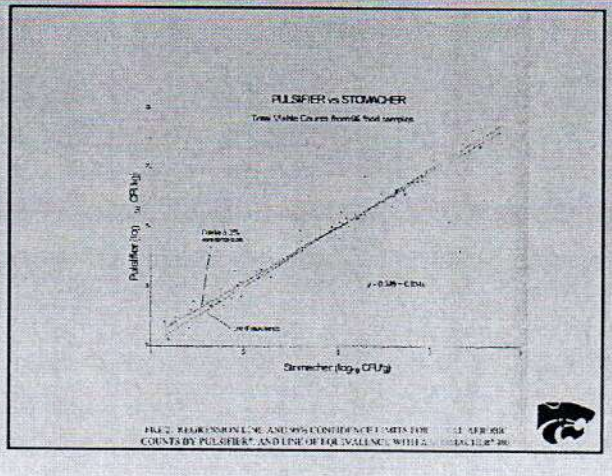
ATP tests 10 – 20 minutes

Advances in Total Viable Cell Count Methodologies

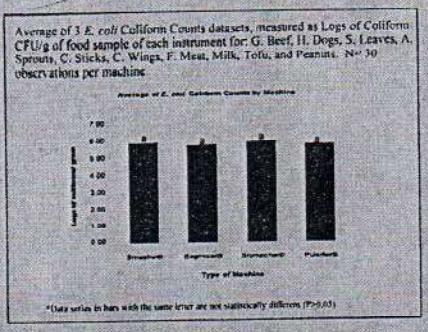
- Stomacher versus Pulsifier
- Petrifilm, Redigel, ISOGRID, and Spiral Plater data
- Fung's Double Tube for 6 hr *Clostridium perfringens* enumeration

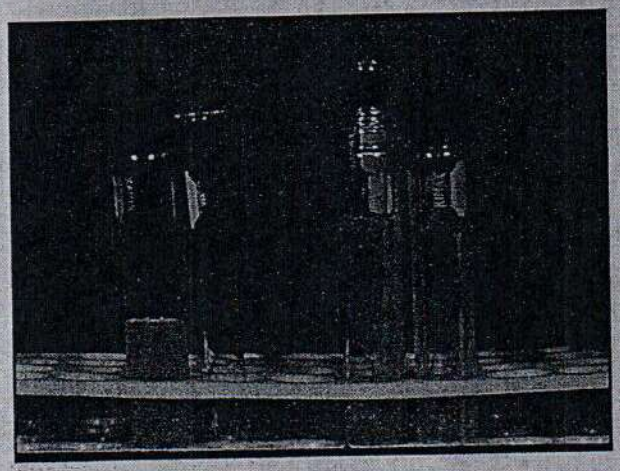






Smasher® by AES Chemunex





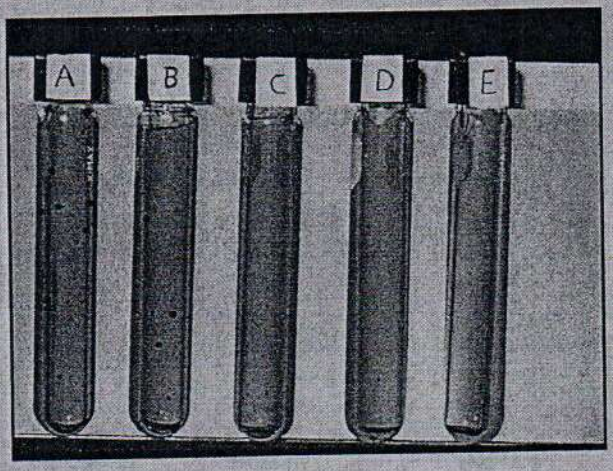


TABLE 3.
FUNG/FUJIOKA SCALE FOR BEACH WATER POLLUTION BASED ON SINGLE SAMPLE CONCENTRATIONS (cfu/100 mL) OF *CLOSTRIDIUM PERFRINGENS* USING THE FUNG DOUBLE TUBE (FDT) METHOD

Pollution category	FDT (cfu/10 mL)*	Extrapolated FDT(cfu/100 mL)	Scale of beach pollution
I	0	<10 cfu	Uncontaminated
II	1–10 cfu	10–100 cfu	Nonpoint contamination
III	11–50 cfu	110–500 cfu	Sewage contamination
IV	>50 cfu	>500 cfu	Elevated sewage contamination

*After confirmation with conventional method.
cfu, colony forming units in Shahidi Ferguson Perfringens agar medium at 42C in 6 h.

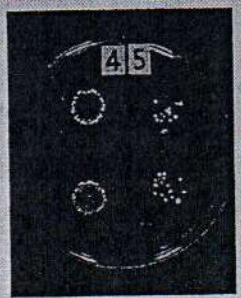


Figure 1. Duplicate rows of different dilutions from a soil sample. The numbers 4 and 5 represent 10^{-4} and 10^{-5} dilutions, respectively. Data obtained from the 10^{-4} dilution were used to calculate cell density.

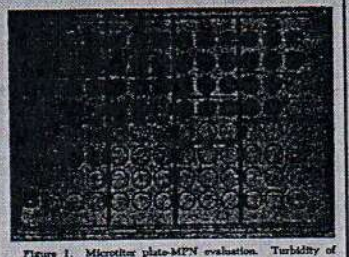
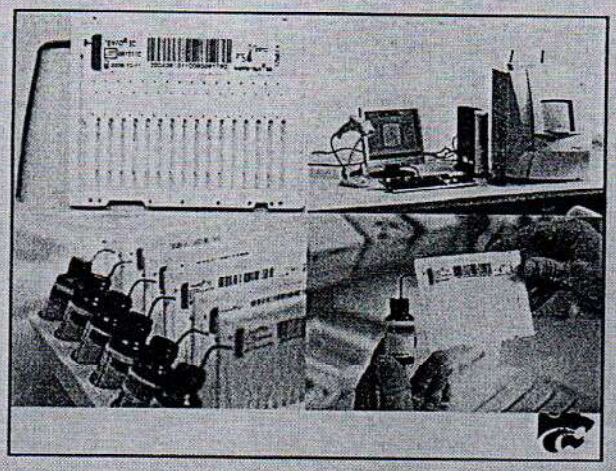
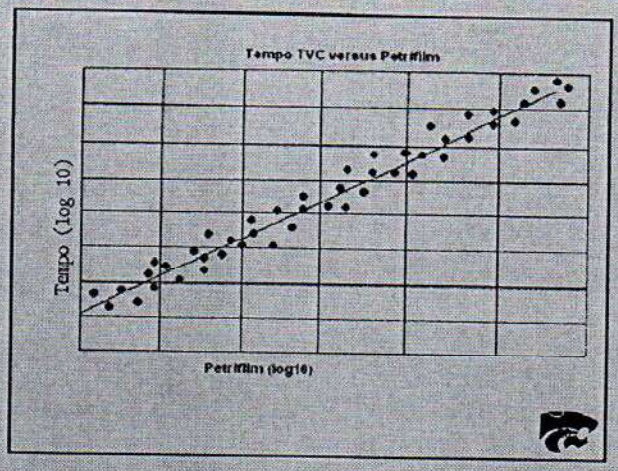


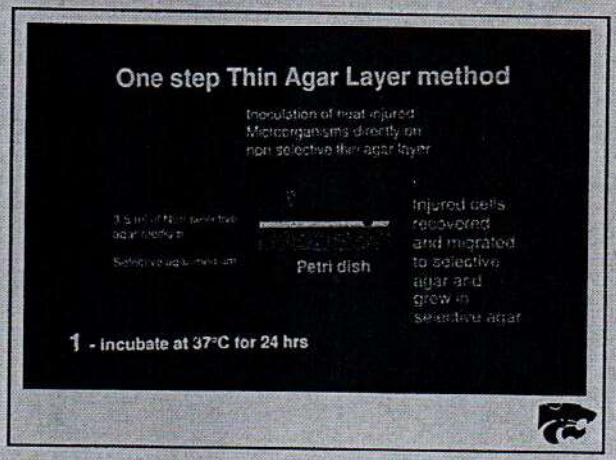
Figure 1. Microtiter plate-MPN evaluation. Turbidity of the wells indicates growth. MPN of sample A is obtained by multiplying 45 (from table 1; 3+/3, 1+/3) x 4 x 10^{-5} or 1.8 x 10^{4} organisms/ml.

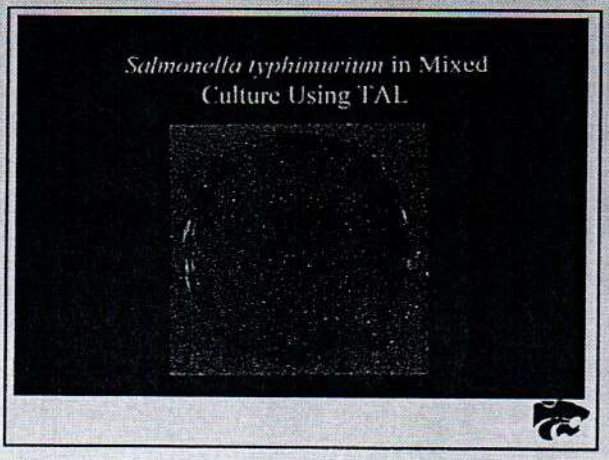


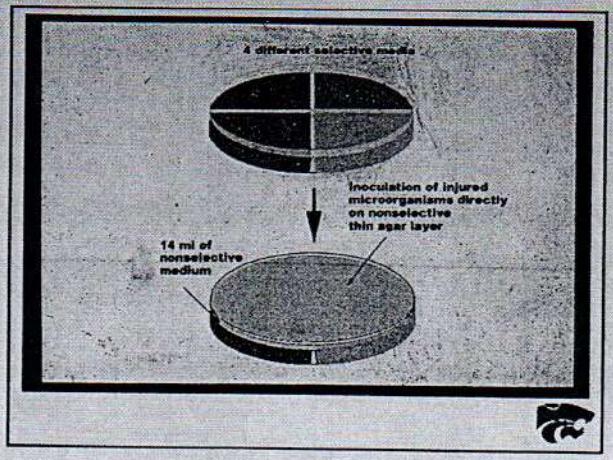


Kang and Fung

THIN AGAR LAYER METHOD FOR THE RECOVERY OF INJURED CELLS IN FOODS AND ENVIRONMENTS







Instantaneous Results–Seconds

- Catalase and Enzyme Tests
- Food Residual Tests
- Biosensor

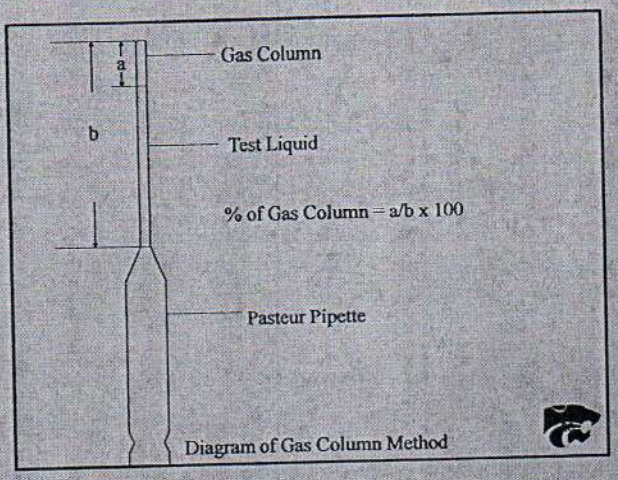
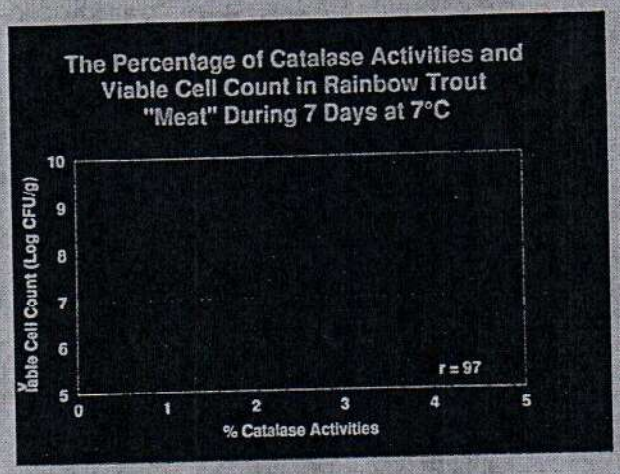
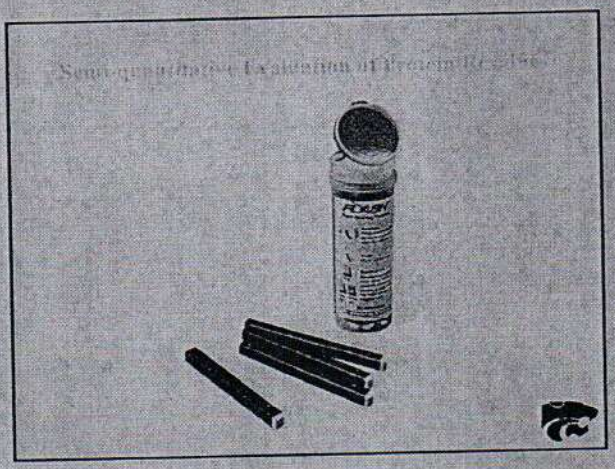


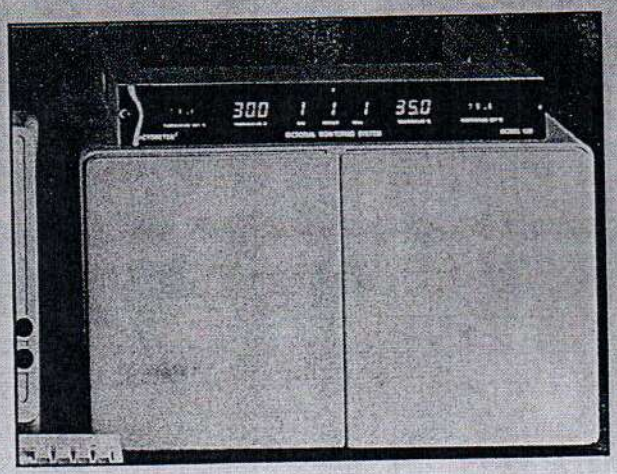
Diagram of Gas Column Method

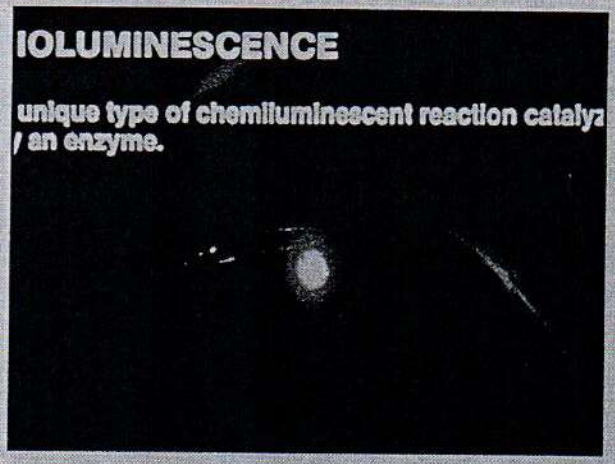


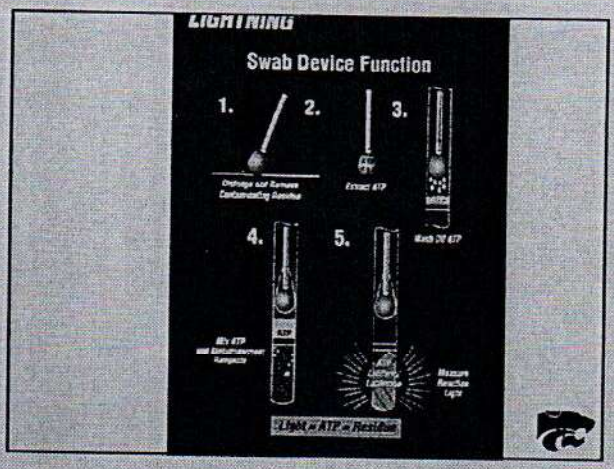


Automated Instruments Can Monitor Microbial Activities With Ease

- Conductance-Malthus
- Impedance-Bactometer, RABIT
- Bac T/Alert
- Omnispec
- BioSys

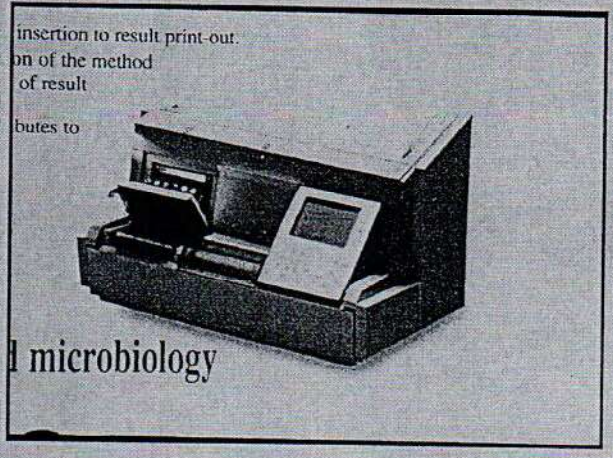


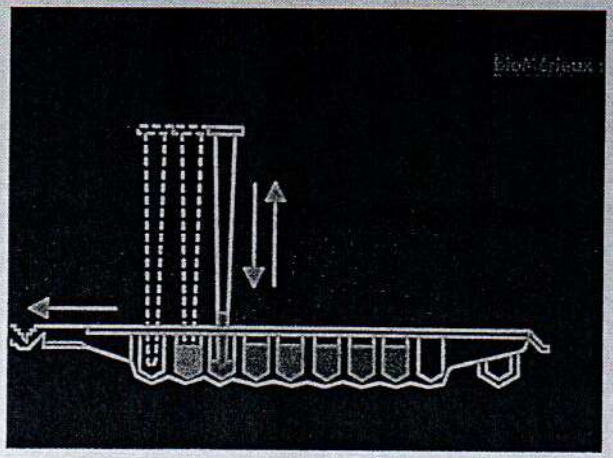


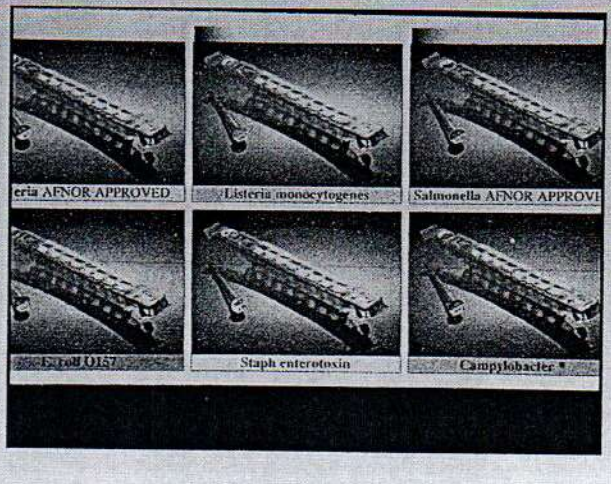


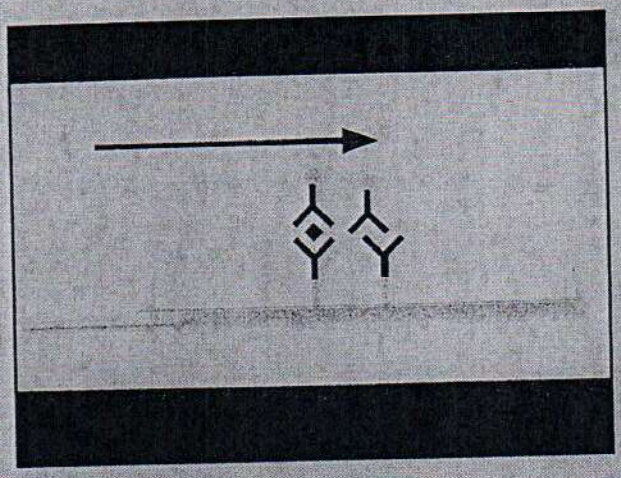
Advance in Immunological Testing

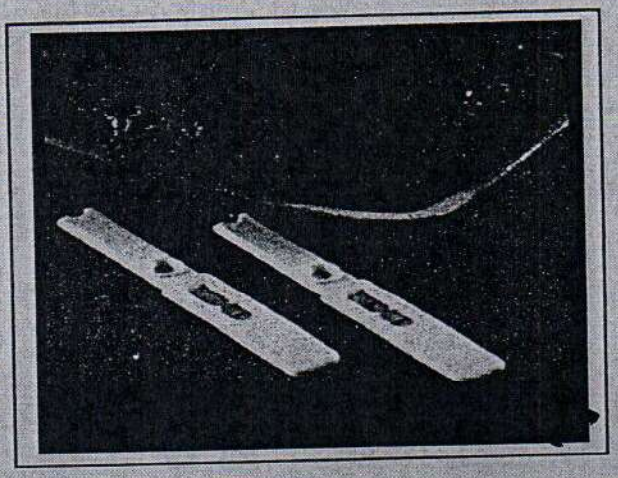
- ELISA Tests, VIDAS
- Diffchamb, Detex System
- Lateral Migration immunoassays
- Immunomagnetic Separation Technologies

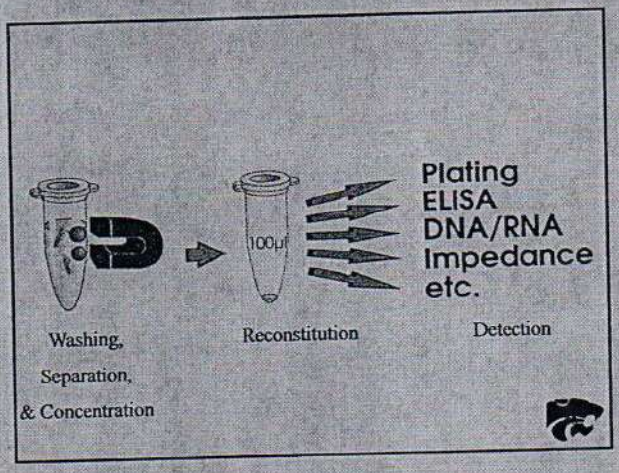


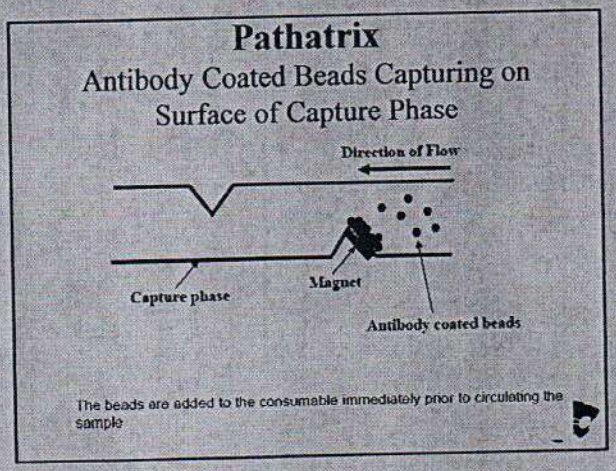


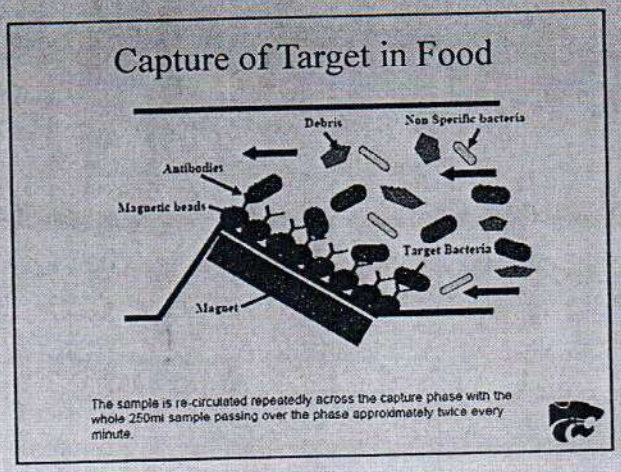


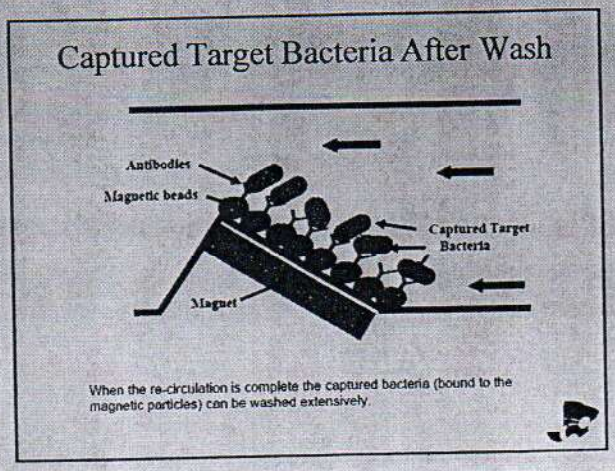





100µl
Plating
ELISA
DNA/RNA
Impedance
etc.
Washing,
Separation,
& Concentration
Reconstitution
Detection


Pathatrix
Antibody Coated Beads Capturing on Surface of Capture Phase
Direction of Flow
Capture phase
Magnet
Antibody coated beads
The beads are added to the consumable immediately prior to circulating the sample


Capture of Target in Food
Debris
Non Specific bacteria
Antibodies
Magnetic beads
Target Bacteria
Magnet
The sample is re-circulated repeatedly across the capture phase with the whole 250ml sample passing over the phase approximately twice every minute.

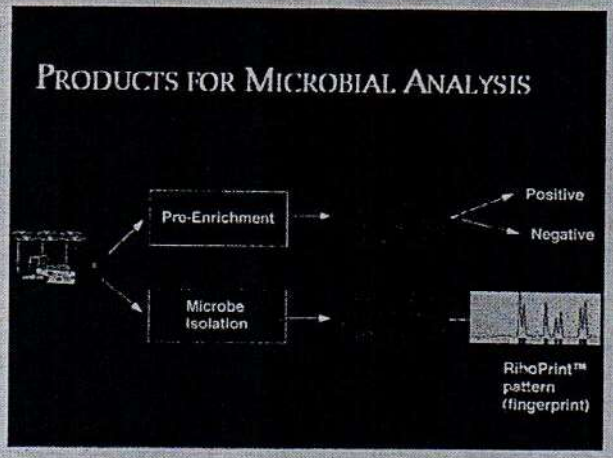

Captured Target Bacteria After Wash
Antibodies
Magnetic beads
Captured Target Bacteria
Magnet
When the re-circulation is complete the captured bacteria (bound to the magnetic particles) can be washed extensively

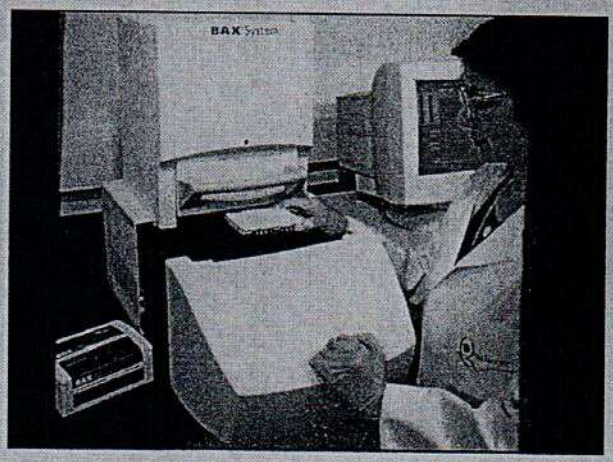
Current State of Microbiological – Genetic Tests

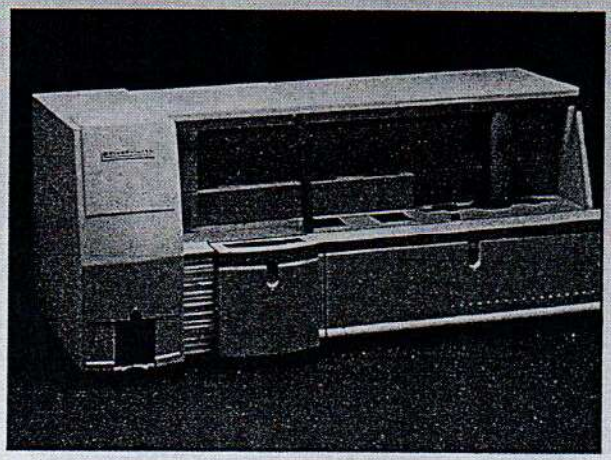
- DNA/RNA Hybridization
  - Needs 6 log CFU/ml, g, cm² for reaction
- Polymerase Chain Reaction and Related Technologies
  - Needs Enrichment to Ensure Monitoring of Viable Cells and Dilute Inhibitors
- Microarray, Biochips, Proteomics, Geonomics
  - Needs Sample Preparation Before Application
- Biosensors
  - Needs Concentration of Target Cells Before Detection

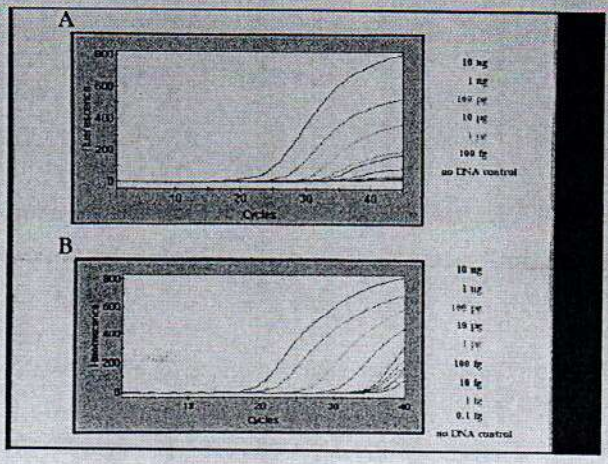
Genetic Methods

- DNA/RNA Hybridization
- PCR –BAX
- Molecular Beacon Technology
- Probelia
- Riboprinting and Pulse Net Systems

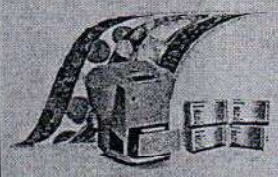








Thermo Scientific SureTect Real-Time PCR System



- Designed to quickly and accurately detect microorganisms in a broad range of foods samples
- Assays available now:
  - *Salmonella* species (AOAC-RI approved)
  - *Listeria* species
  - *Listeria monocytogenes*

Why choose SureTect?

- **Ease of use** -pre-filled lysis tubes and tableted PCR reagents minimize the number of pipetting steps and hands-on time
- **Speed** – optimized single enrichment step for key food matrices and straightforward sample lysis in less than 20 minutes
- **Convenience** – common PCR protocols facilitate efficient processing of multiple assays in the same run
- **Superior PCR technology** – probe-based real-time assays for unparallel sensitivity and specificity

It's easy to be sure!

For more info on the SureTect System please visit the Thermo Scientific booth TODAY!

Advances in Biosensors

- Microarrays, Biochips
- Nanotechnology
- Sampling clean-up and extractions
- Viability and sensitivities of cells

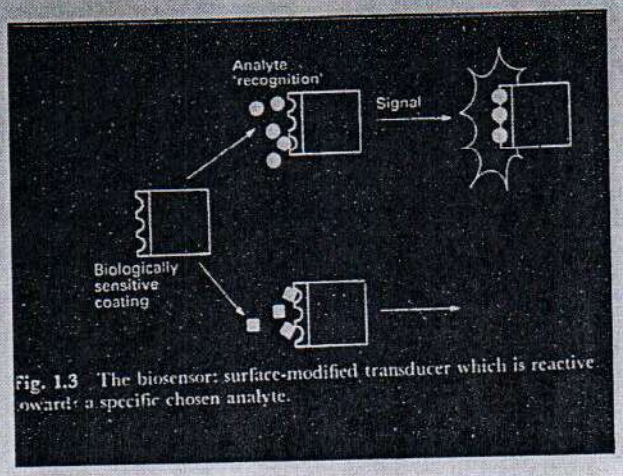
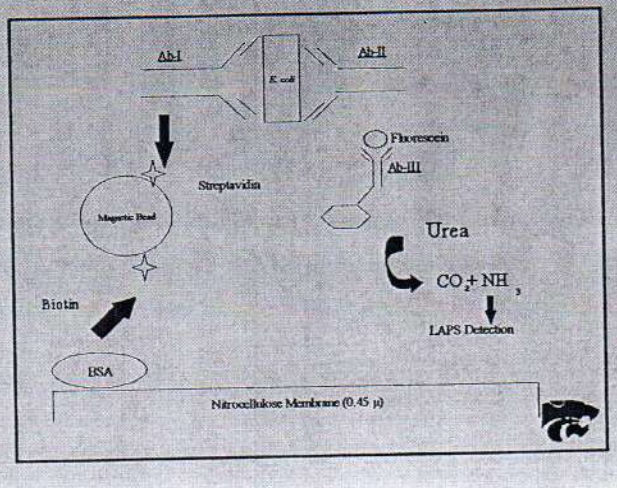


Fig. 1.3 The biosensor: surface-modified transducer which is reactive towards a specific chosen analyte.



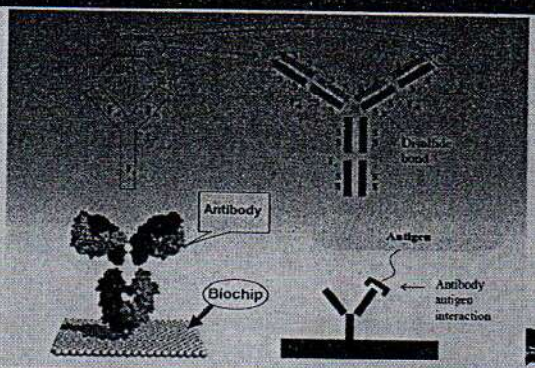
Nanotechnology: Why Size Matters

Gold nanoparticles can emit intense heat

A cluster of gold nanoparticles 50 nanometers in diameter created a much larger crater in the ice sample.

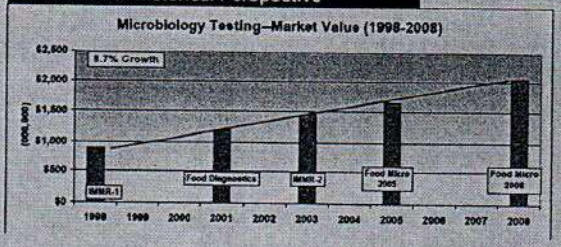
www.physorg.com/printnews.php?newsid=63003999

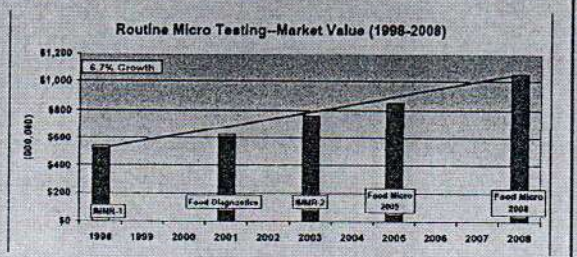
Microbial Nanosensors on a Chip

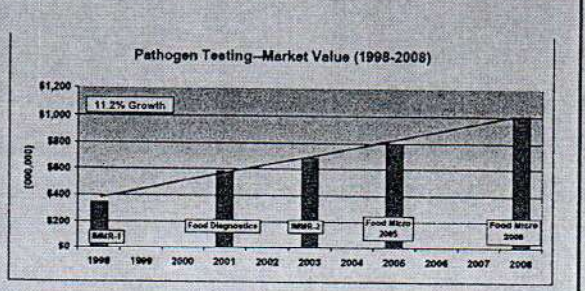


Food Plants with >25 Employees	**40,000**
Tests/Plant/Year	
Routine	15,005
Pathogen	3,453
Total	18,458
Total Tests (Millions)	
Routine	600.2 M
Pathogen	138.1 M
Total	738.3 M
Market Value ($$ Million)	
Routine	$1,050.0 M
Pathogen	$1,007.4 M
Total	$2,057.4 M

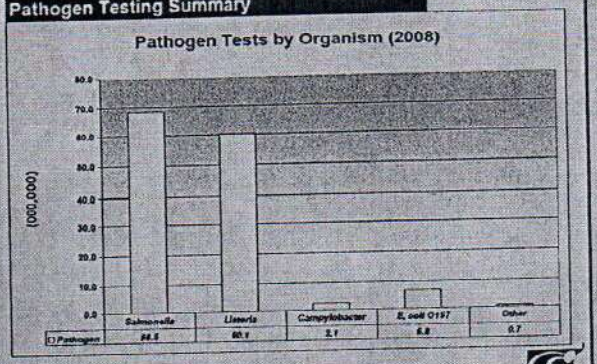
Market Value--Historical Perspective

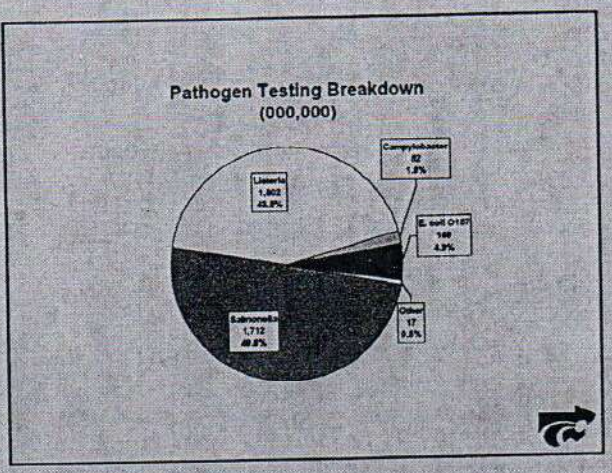




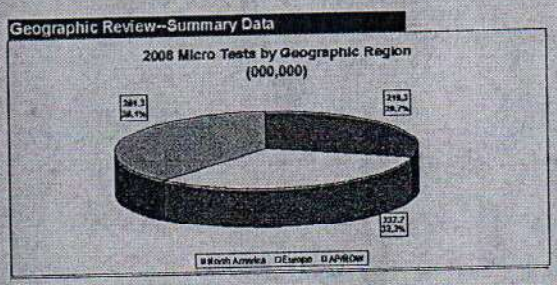


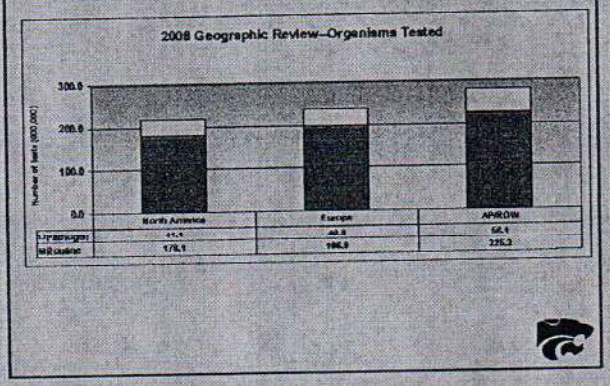
Pathogen Testing Summary



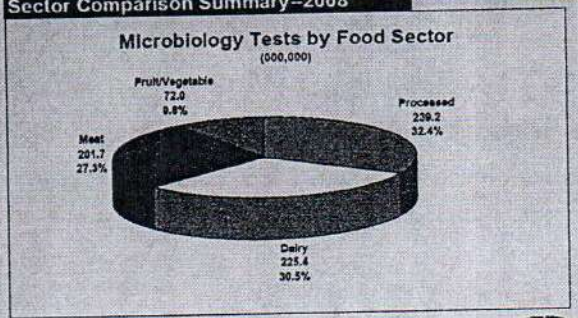


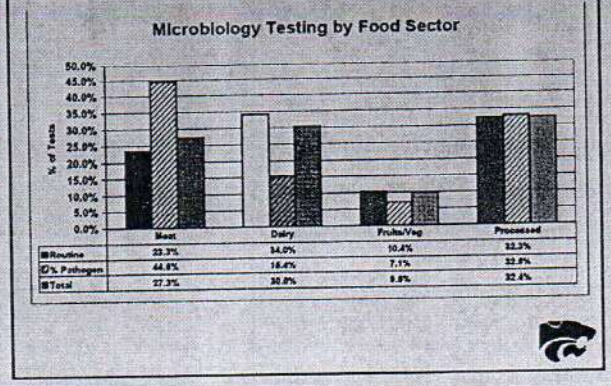
Geographic Review--Summary Data



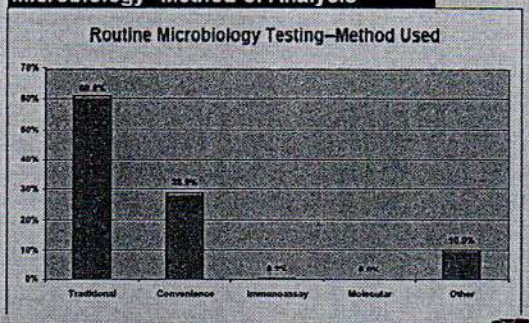


Sector Comparison Summary--2008





Microbiology--Method of Analysis



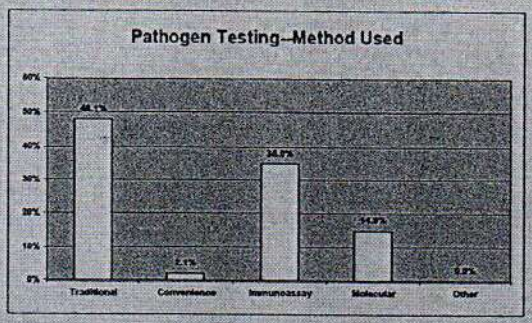


TABLE 2. *Comparative analysis of ground beef, ground pork and raw milk (Plated on APC, Redigel, Petrifilm, Spiral Plate and Isogrid) by Pearson correlation coefficient.*

Method	APC	Redigel	Petrifilm	Spiral P.	Isogrid
APC	1.000	0.999	0.999	0.999	0.999
Redigel	0.999	1.000	0.999	0.999	0.999
Petrifilm	0.999	0.999	1.000	0.999	0.999
Spiral P.	0.999	0.999	0.999	1.000	0.999
Isogrid	0.999	0.999	0.999	0.999	1.000

TABLE 6. *Total cost analysis per plate (Per viable cell count).*

Method	Material and media cost	Labor costs	Total cost
APC	$2.06 (12.36)	.21 (1.26)	2.27 (13.62)
Redigel[2]	1.16 (6.96)	.21 (1.26)	1.37 (8.22)
Petrifilm[3]	1.16 (6.96)	.21 (1.26)	1.37 (8.22)
Isogrid[4]	3.01 (3.01)	.32 (.32)	3.33 (3.33)
Spiral Plate System[4]	2.06 (2.06)	.21 (.21)	2.27 (2.27)

Notes:
[1]Does not include initial cost of equipment (Spiral Plate System ranges from $11,700 to $12,500 including the plater, vacuum system, and colony counter; Isogrid ranges from $2,500 to $4,000 including the time counter, vacuum system, 12 filter heads, 3 clamps, and 100 filters. Approximate costs as of 3-1-88).
[2]Cost per plate is reduced by quantity purchase.
[3]Does not reflect possible enzyme pretreatment before filtration-cost averages 30 cents per sample for enzyme treatment.
[4]Assumes an average of six plates for one viable cell count and necessary dilutions.

RAPID MICROBIOLOGICAL METHODS AND DEMONSTRATING A RETURN ON INVESTMENT: *IT'S EASIER THAN YOU THINK!*

BY MICHAEL J. MILLER
PRESIDENT, MICROBIOLOGY CONSULTANTS, LLC
***American Pharmaceutical Review* Vol 12 Issue 5**
July/August 2009. Pp 42-47
Russell Publishing Company, Indianapolis, MN.

Example of Operating Costs for the Conventional Method (CM) and the Rapid Microbiological Method (RMM) for Airborne Particles

	CM	RMM Year 1	RMM Year 2
Number of tests per year	70,000	14,000	14,000
Total sampling, testing, data handling and documentation resource time per test (hours)	1.00	0.10	0.10
Calculated annual labor (hr)	3,500,000	70,000	70,000
Total Annual Costs	$ 3,675,000	$250,000	$ 466,000

CM used agar base technology. **RMM** used Mie-scattering technology which can detect, size and quantitate both viable and nonviable particles
Miller, Michael J. 2009. Rapid Microbiological Methods and Demonstrating a Return on Investment :It's Easier Than you Think. *American Pharmaceutical Review*. Vol 12 Issue 5 July/August 2009. Pp. 42-47. Russell Publishing Company, Indianapolis, MN.

Predictions (1995)

1. Viable Cell Counts Will Still Be Used
   a. Early sensing of viable colonies on agar 3-4 hrs
   b. Electronic sensing of viable colonies under microscope 2-3 hrs
   c. Improvement of vital staining to count living cells
   d. Early Sensing of MPN

   2008 (+) on Target

Predictions (1995)

2. Real Time Monitoring of Hygiene Will Be In Place
   a. ATP
   b. Catalase
   c. Sensors for biological materials
   d.Sensors for chemical materials

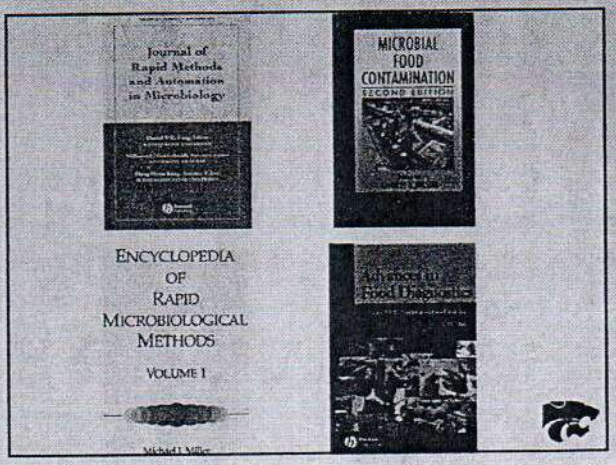
   2008 (+) on Target

Predictions (1995)

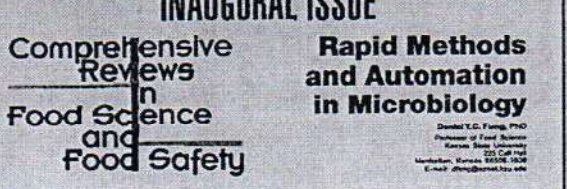
3. PCR, Ribotyping, Genetic Tests Will Become Reality in Food Laboratories (+)
4. ELISA and Immunological Tests Will Be Completely Automated And Widely Used (+)
5. Dip Stick Technology Will Provide Rapid Answers (+)
6. Biosensors Will Be In Place In HACCP Programs (?)

Predictions (1995)

7. Microarrays, biochips, nanotechnologies will be widely used. (+)
8. Effective Separation and Concentration of Target Cells Will Greatly Assist Rapid Identification. (+)
9. Microbial Alert Systems Will Be In Food Packages (+/?)
10. Consumers Will Have Rapid Alert Kits For Pathogens At Home (?)



Fun Fung Fact:



As of March 2005 the Webstie of Fung's paper received 2,967 individual 'hits'!