ROUTINE LABORATORY PROCEDURES AND SAMPLE LOADING

1. Are there viable organisms present?
2. If so, is their concentration important?
3. Are the kinds of microorganisms important and/or necessary to identify?
4. How complete an identification is needed?
5. What are the characteristics to be assessed: Biochemical, physical, immunological, morphological, antimicrobial, susceptibility, genetic makeup, etc.?
6. Can "surrogate" microorganisms be used in place of pathogens of interest in validation procedures (acid tolerance, heat resistance, or other relevant characteristics)?
7. Am I looking for a special metabolite, compound, or end product before, during, or after a period of growth/no growth?
8. How often do I need to test?
9. How many samples do I process at the same time?
10. Will instruments be more useful, faster, cheaper or more accurate for my needs than manual methodology?

I. ROUTINE LABORATORY PROCEDURES

A. Media Preparation-Robotics in General

1. Solid Media
   - A. Plate pouring, stacking (Agarmatic, New Brunswick Scientific Edison, NJ, Vista Technology).
   - B. Integra culture flasks with membrane and compartmentalized technologies (Cole Porter Co.).
   - C. Promega uses paramagnetic particle technology in an automated procedure to isolate RNA in 96 well microtiter plates (MagniSil total RNA mini-Isolation System).
2. Liquid Media
   - A. Non-dedicated can be uses with own plates, tubes, etc. (Hamilton, Tecan, Biomek, Zymark, Cetus, Dynatech). Metrohm-Peak autosample MultiProbe II Plus (semi adaptable) Perkin Elmer.
   - B. Dedicated must be used with manufacturer's reagents and labware (Abbott, Perkin Elmer)
3. Microtitier plate centrifuge. Velocity 11 markets the BenchCel Microplate Handling System and V Spin Integrated Microplate Centrifuge.
4. Mircoplate Washers/Dispensers (BioTek) and R-Series Bench Cel Velocity II (by Velocity).

B. Diluting

1. Liquids:
   SPD 3000 (Dynate · .), Microlab AT Sample Transfer System and Microlab Nimbus (Hamilton), Biomek (Beckman), DuPont, Spiral Biotech, Inc. The Biomek now has a high density replication system (1536 – 3536 colonies/8 x 12 cm filter) with multiple uses added to its robotic workstation. Whatman (DNA binding) filter plates. Electronic Pipettors, Matrix; Brand Technology Scientific (repeating pipette), Millipore System, and the Eppendorf epMotion 5075 liquid handling

workstation for SV96 nucleic acid purification systems (Eppendorf provides reagent dispensing, etc.).

2. Solid and Semi-Solid Materials
   Gravimetric Dilutor (Spiral Biotech).
3. Dialysis Cassettes
   Disposable Slide-A-Lyser Dialysis Cassettes (0.1-12 ml capacity) of different membrane molecular weights (Pierce).

C. Plate Counting

1. Colonies
   Imaging Systems (Artek), Petri Plate Reader (Spiral Biotech, Inc. Instruments), Automated Microbiology System (Biofoss).
2. Cells in Liquid
   DEFC 40-1 (Optomax, Inc.) Epifluorescence (Artek Omnicon 2000), See also particle counting. Nexcelon Bioscience's Cellometer P2 counting chamber for mammalian cells.
3. Plate readers for microtiter plates of various sizes adapted for fluorescence, luminescence, immunoassay, biochemical analysis (many companies, e.g. Molecular Devices SpectraMax L, BioTek Instruments "Synergy 4" has a multi-detection Microplate reader, which includes fluorescence intensity, luminescence, fluorescence, time-resolved fluorescence polarization, and UV-visible absorbance. Beckman Coulter's "PARADIGM Detection Platform" is a modular system that reads similar types of fluorescence (6- to 1536-well plates). Perkin Elmer has a new "EnVision Multilabel Plate Reader." There is also Promega's Glow Max Detection Systems. 3M markets the "RAMP" technology, rapid detection reader for their "lateral flow immunoassay platform fluorescent test for Influenza A and B Antigens (15 minute test) ThermoFisherSci launches NanoDrop 2000 spectrophotometer (UV-visible) measuring as low as 0.5 µL sample size: (DNA, RNA, and Protein). Giles Sci. Inc. has the "BIOMEC V3 Microbiology System" which automates reading and interpretation of microscan (Siemens) and sensitive (TREK) mic and ID panels.

D. Staining

Automatic staining to microscopes slides, "Code-On" robotic handling system (Fisher), LKB (for electron microscope), Tomtek, Aero-spray, Wescor (Logan, UT). Use of vital stains to distinguish viable bacteria (BacLight). EMIGL Automated Immunogold Labeling System for tissue and cell samples (Leica Microsystems).

II. PHYSICAL METHODS OF DETECTION (See Also IV Biosensor Systems)

A. Particle Counting, Flow Cytometry

Electrostatically charged microorganisms selectively counted and/or recovered. Coulter Scientific Instruments, FL. Flow cytometry (EPICS XL, and Gallios: Coulter Corp.) FACS Scan (Becton-Dickinson), BD Biosciences markets the BD Influx Cell Sorter. Coulter also has a laser LS Series for diffraction particle sizing and the Delsa Nano, which uses multiple angle light scattering. Malvern Instruments, "Zetasizer

Nano", particle sizing (e.g. Cadbury-Chocolate Cocoa beans ground properly. Teledyne Tekmar Apollo 9000 can be used for particle analysis of wastewater, industrial effluents, drinking water, brines, etc. It will report total organic compounds and total nitrogen simultaneously using a chemiluminescent detector and a flexible ranging furnace. Watt Technology's Dawn Heleos measures macro molecules. Also see the Nicomp Sub-micron Particle size Analyzer (Particle Sizing Systems Co.). The Laser Ion Mobility Spectrometry (LMIS) portal demonstrator collects chemical substances (gases, drugs, explosives, etc), in the air, charges, ionizes, sorts by mobility to determine the particle chemical signature (EADS North America Co.) See also GC/MC; LC/MS for combinations.

B. Image Analysis

Video camera connected to an image array micro-processor/computer, Optical Information: Image is digitized and converted to pixels (Zeiss, Biaquant) also MI-100 Interpreter for counting ISO-Grid HGMF. Interpret biochemical tests and growth patterns. Applied Imaging (AI) (Genetix). Ariol Automated Imaging Analysis protein biomarkers. The Innothech IS-1000 digital imaging system combines CCD (Charge Coupled Device) Video Camera Technology for low cost instant photography, image enhancement and processing. Images can be imported into word processing and desktop publishing software. Role of image analysis system in quantitating molecular weight and antibody titers. Leica Microsystems has similar imaging workstations (Q 550 IW, Quin). Bio-Rad markets a VersaDoc Model 4000 with multi-mega pixel ($3.2x10^6$ pixels) resolution for colorimetric, fluorescent, chemiluminescent and chemiluminescent detection. Syngene's G:BOX ChemiXT16 automated chemiluminescence and fluorescence imaging analysis systems. Carestream Molecular Imaging markets the Kodak Image Station MM Pro also features CCD camera technology and detects all ranges from visible to x-ray detection. Kodak has a new dye: Kodak X-sight with a higher sensitivity. It can be used with instrumentation other than Kodak in vivo imaging systems and Kodak Imaging stations. This dye has a fluorescence-near infrared emissions. Photodyne has a multi-wave gel imaging system. Kodak imaging systems are now licensed by Carestream Health, Inc.

C. Chromatography

Method based on differential characteristics of separate components partitioned between two phases, one phase stationary, and the other mobile. Detectors (ionization, flame, electronic capture, chemiluminescent, etc.) monitor effluent. Stationary phase can be a solid or a liquid. For ID of anaerobes, volatile compounds, environmental pollutants, unusual fatty acid components, mycotoxins, endotoxins, other microbial ID. Increased sensitivity via selective ionization now available. Chromatography instruments can, and often do, feed into a mass spectrometer.

1. **Thin Layer Chromatography.** CAMG automates it with MS
2. **Gas Chromatography** (also High Speed GC, Capillary GC)
   Solid columns (carbonwax polysiloxane). Automated (Hewlett-Packard, "HP"). Mobile phase is a gas, Microbial Identification Systems uses "HP" automated GC ("MIS"). Cell wall fatty acid analysis ID of microorganisms by comparison with

computer data back "libraries" established and upgraded by company. Two dimensional GC allows higher separation (Thermo Electron Corp's Thermo TRACE 2D GC), Hercules Electronic Nose (Alpha M.O.S.), also a portable version, uses ultra-fast GC. NIST now has a GC, GC-MS library of 44,008 compounds (See Scientific Instrument Services.

3. **Liquid Chromatography** (High performance or high pressure liquid chromatography (HPLC), ion exchange, LC affinity chromatography, Revered Phase LC (RPLC) (Agilent). Perkin Elmer has a new UHPLC, "Flexar" with new software, "Chroma". NanoLC Ultra for nanochip work by EKSigent separates complex protein mixtures. Dionex's automated UltiMate 3000 RSLCA 5 minute finished validation report. CVC Technologies has a new Atima (15,000 psi rated) with a 3QMS option, and also a nano-XPLC with the 3QMS. Shimadzu has a compact LCMS2020 with UF scanning technology. UHPLC is ultra high-performance liquid chromatography which utilizes sub-2μm-diameter stationary phase particles to provide improved performance over conventional HPLC.
4. **Air Analysis** CDS Analytical, Inc. has a Dynatherm 9300-a thermal desorption systems sampling several liters of air to detect compounds (such as chemical warfare agents). MetrohmAG introduced a particle into liquid sampler to collect aerosols, feeding them into an ionchromatograph.

D. Mass Spectroscopy (MS, GC/MS)

This is mainly a qualitative technique. This instrumentation has increased uses in proteomics to identify and characterize proteins in cell lysates, isolated organelles or purified multisubunit complexes. Electron beam or charged reagent gas ionizes the sample. Resultant fragmentation pattern indicated molecular structure and ID. Usually in conjunction with GC, LC, electrophoresis, HPLC, etc. Laser ion mass analyzer. Automated (Hewlett-Packard). Current applications for MS in Microbiology rely on the ability to register biomarker ions in a broad mass/charge range to present a unique and characteristics profile of individual microorganisms-initial pyrolysis has also been used. MALDI-TOF means Matrix-Assisted Laser Desorption-Ionization Time-Of-Flight. In MALDI-TOF techniques a matrix of small organic crystals is irradiated by a laser pulse under a 20kV voltage. The produced ions form a gas-phase matrix, which is transmitted by a mass analyzer to generate a mass spectrum. The mass/charge ratios of the ions are related to the time it takes to fly through the MS flight tube. TOF mass analyzers have been used to detect intact microorganisms for more than a decade. MS Electrospray Ionization (ESI) has also been used for nutraceuticals. The addition of DART (Direct Analysis in Real Time) ion source to JEOL's AccuTOF instrumentation allows quantification of small molecules without sample preparation. MS has been combined with bioaffinity surfaces (antibodies, antigens) and also used to screen drugs for bacterial contaminants, food safety testing, and to discriminate between antimicrobial resistance and sensitivity in *Staphylococcus aureus*. Varian Inc. markets the 4000 GC/MS. This instrument features quadruple technology allowing the automatic selection of either electron ionization (EI) or Chemical ionization (CI). It can be coupled with Tandem MS/MS detection yielding spectra for compounds indiscernible in conventional reaction modes. Agilent Technologies markets a nano-LC-MS for proteomics phosphopeptides and also markets Agilent 5973, 5975, and

700A inert GC/MS systems with a solid inertion source for forensic environment and FOOD SAFETY applications. CDS Analytical's model 5000 Pyroprobe allows complex polymeric materials to be analyzed by GC and GC/MS (can analyze contaminants, solvents, and additives) at set temperatures up to 1400° C. Shimadzu's GCMS-QP2010 provides analysis of difficult samples and trace components. Fast GC-MS JEOL uses a combination of Agilent GC and JOEL AccuTOF/MS. Varian also markets the first Fourier Transform/MS for structure/characterization of proteins and other iological molecules for "top-down" proteomics. This eliminates the need to digest large proteins into smaller pieces before MS analysis. Perkin Eimer, INC. has a Turbo Matrix Headspace Sampler for volatile compounds, which extracts analytes from water and other matrices prior to injecting into a GC (for food packaging companies and pharmaceutical companies needing constant QC). Gyrolab MALDI, a "CD" disc concentrates and purifies protein digests and crystallizes them directly on the "CD," which is then placed into a MS for sample analysis (96 samples simultaneously). Other fast MC detectors for LC by Waters, Shimadzu and ThermoFisherSci's with Orbitrap analyzer. These increase sensitivity by 25-300%. There are cartridges to detect melamine in foods and beverages which produce a sample for LC-MS or GC-MS. Brucher MicroFLEX MS allows direct application of liquid culture or colonies to the MS.

E. Electrophoresis (Gel Electrophoresis, PAGE, immunoelectrophoresis, PFGE Pulsed Field Gel Electrophoresis)

Particle migration in an electric field depending on surface charge and size. pH gradient may also be involved. Support systems of gels, discs, slabs, and liquid. Microbial fingerprints of patterns searched against data banks. Some automated: Pharmacia's Phast System, Pharmacia Pulsaphor apparatus (PFGE and AMBIS-computer controlled electrophoresis system with a Beta scanning module. Mini-Protean Tetra Systems for mini-vertical gel electrophoresis (up to 4 SDS-PAGE simultaneously). Cappillary gel electrophoresis uses fused silica columns (75 µm) filled with linear polyacrylamide. Molecular sieving occurs of DNA species as they migrate through the gel. An automated instrument, High Performance Electrophoresis Chromatography (HPEC) (Applied Biosytems, Foster City, CA) combines Electrophoresis with Chromatography for DNA sequencing by capillary electrophoresis. Millipore Co. BioImage electrophoresis and image analysis combination and "2-D" electrophoresis software systems. Capillary Electrophoresis (CE) has been used to analyze low concentrations of metabolites (see "Metabolomics") in biological fluids and cellular extracts. Capillary sieving electrophoresis has been used in metagenomic studies. CE-MS has been used in applications to bacterial metabolomics (*Campylobacter* etc) Anal. Chem. 2004 76:619-626). Biorad's Experion automated electrophoresis system uses biochip technology (see mic · arrays and microfluidics section) Micronit Microfluidics supplies micorf · idic chips for capillary electrophoresis. ProRegXL Tool is a new tool suite designed to rapidly regroup a large number of identical electrophoretic profiles, control gel quality, determine signal attenuation and draw pie chart. (B. Massias and M.C. Urdaci BioTechniques 46:441 (2009).

F. Calorimetry (Flow, Differential, Batch)

Analysis and measurements of heat produced or absorbed in proportional to the rate of the process and/or concentration of ingredients. Heat production or absorbance measured by voltage changes via thermocouples. Susceptibility or resistance to inhibitory agents, thermogram characteristic profiles or microorganism for ID quantification of populations and growth kinetics. Automated Differential Scanning Calorimeter DSC-4 (Perkin Elmer) Robotic-System, Polyhedron Laboratories isothermal calorimetry used to assay protein dissociation constants.

Measurement Technique	Special Measurement Modes	Use
DSC	TM DSC (ADSC, IsoStep)*	For automated optimized temperature resolution of neighboring mass changes.
TMA/DLTMA	∞ Dilation (low load sample) ∞ Penetration (large load on sample) ∞ Tension ∞ Bending	Mode to measure the coefficient of thermal expansion Particularly suitable for the analysis of thin films (glass transition, melting temperature, shrinkage) Glass transition of filled materials and other stiff samples
DMA	∞ Tension ∞ Compression ∞ Shear ∞ Bending	Above all for fibers and thin films Foams, elastomers Elastomers, most thermoplastics powder, pastes, Fiber-reinforced plastics, thermoplastics, thermosets

* **Mettler – Toledo, Inc.** (Greifensee, Switzerland).

Special measurement modes for different TA techniques and their applications.

DSC: Differential Scanning Calorimetry
TGA: Thermogravimetric Analysis
TMA: Thermal Mechanical Analysis
DMA: Dynamic Mechanical Analysis
SDTA: Single Differential Thermal Analysis

G. Photometric (See Biochemical, Immunologic Detection and Biosensor Sections)

H. Luminescence (Bioluminescence, Chemiluminescence, Continuous Luminescence)
See Microplate readers for luminescence

Electrons absorb energy and are raised to a higher energy state (usually unstable). Excess energy can be emitted as photons as the electrons return to ground level. Biomass measurement based on ATP, reduced flavin mononucleotide (FMN), NAD, metal concentration. Phagocytosis, immunological reactions, enzyme reactions, glucose analyses, vitamin and nitrogen determination, microbial content of soils, bacterial contamination of water and food, organic pollution, drug potency, sludge activity, toxins (Luminometers, Corning, Perkin Elmer Co. "EnVision", Molecular Devices LMax II, Turner, etc.). Millipore has an automated system for rapid accurate detection of microorganisms, the Milliflex based on ATP and sensitive to CFU/sample detection levels. Molecular Devices SpectraMax LE.

1. **Bioluminescence**
   A. *Vibrio harveyi*: "lux AB" bacteria luciferase genes fused into *Escherichia coli*: luminescence stimulated by aluminum, etc. – environmental monitoring.

   B. ATP using firefly luciferase and luciferin ATP. One bacterium = 2.5×10^{-10}ng; 1×10^{3} bacteria have 10^{-13}g ATP (detection limits of assays). Firefly luciferase (E)+ reduced luciferase (LH_2)+ATP+ELH_2AMP+PP $ELH_2AMP+O_2 \rightarrow E+AMP+CO_2$+ **Light Photons @550nm**+ Thiazolidine

   C. Luciferase (E) +FM NH_2 (reduced flavin mononucleotide) + EF $MNH_2+O_2 \rightarrow$FMN EFMNH_2+O_2+RCHO (aldehyde) → E+FMN+RCHOOH+**Light Protons @492nm.**

   D. Luciferase (E) +NADH-NADPH+aldehyde= Excess FMN →FMN→ $FMNH_2$; $FMNH_2$ then binds to luciferase (B above.)

   E. BRET: Bioluminescence Resonance Energy Transfer in marine organisms induces GFP Fluorescence via luciferin-luciferase reactions. Various combinations can be used as probes in assessing protein to protein interactions in living cells.

   F. Luciferase has been coupled to a fluorescent protein that facilitates luminescent imaging at the single cell level. (Miyawaki, A. Nat. Methods 4(8): 616-17. 20 1)

   G. RLUC-mediated bioluminescence. Here, a coelenterazine substrate is degraded, resulting in green fluorescence (535-550nm) acting as a reporter probes in living cells. LUC = luciferase (see BRET also). See Merck chemicals group "Mighty Light" Rluc Assay Kit. Luciferase is from *Renilla reniformis*.

2. **Chemiluminescence**
   A. Reduced Luminol (L)+$H_2O_2 \rightarrow$"X"+N_2; X-X_1+**Light Photons @420nm**+H_2O

      X_1 = (aminophthalic acid) →Oxidized Luminol

   B. NADH+ME Blue (ox) →NAD+MB reduced

      MB reduced + $O_2 \rightarrow$ME(ox) +H_2O_2

      The H_2O_2 feeds into A above.

   C. Acridinium esters_OH, $H_2O_2 \rightarrow$ reduced Acridinium + **Light Photons @650-990nm**

   D. Nitrogen Compound R-N, heat (Pyrolysis)-CO_2+H_2O+NO O_3+NO-NO_2+**Light Photons**
   Automated Kjeldahl and nitrogen analyzers (ANTEK)@650-990nm and 1200nm

   E. With excess luminol and H_2O_2, intensity is proportional to metal concentration, N, Fe, Co, Cu, MN, Cr (detection below 1 ppm) Fe, Co most effective.

Luminol Chemiluminescence enhancement for western blots and other immunologic procedures. Pierce Chemical markets a "Supersignal ELISA Femtogram Luminol". Photodynes Inc. (see image analysis systems). Panomics has an interesting "Quatri-gene" system that can even be used with formalin-paraffin embedded supplements. It uses bDNA and qPCR assay. Molecular devices: SpectraMax L 2 and 6 channel. Microplate luminometer can also enable BRET assays

I. Fluorescence

When some compounds are illuminated with light from 200-800 nm, the molecules also become electronically excited and some of the light produced during the return to ground level is emitted at a longer wavelength. If this occurs in less than 10^{-4} seconds, it is termed fluorescence. Fluorescence detectors are available for many instruments. Fluorescence markers for immunological and biochemical test (FITC, MUG, etc.). Sensititre Auto ID System for fermentation reactions (Fluorescence @350nm): indicators only fluo rescence under alkaline conditions, when bond is cleaved enzymatically or loss of fluorescence (e.g. esculin in naturally fluorescent compounds) (see also IV B3 Vidas (BioMerieux Vitek, Inc.). BACTEC 9120 (Becton Dickinson) blood culture 120 vials). Calcofluor White M2R Microtitier plate assay of yeast cell number shows that emitted fluorescence is directly proportional to the number of yeast cells in the wells (*Saccharomyces cerevisiae*). Semiconductor quantum Dots (Qdot's, 2-10nm in size) have the potential to become a new class of fluorescent probes for many

biological applications. Qdot's are nanocrystal particles of Cadmium Selenide (CdSe), Cadmium Telluride (CdTe) or Indium Arsenide (InAs). The size of the spherical core determines the optical properties of the quantum dot yielding narrow emission profiles. Novel surface coatings link the Qdot particles to the biomolecules, they are very photostable. Thus one common excitation source can excite all the quantum dots, giving an almost unlimited array of colors across the visible and into the infrared. There is great sensitivity and photostabilty. Applications include immunocytochemistry, diagnosis, live cell imaging and multiple target analysis. Qdots also available from Evidnet Technologies that are water-compatible, called EviTag crystals, they are supplied as conjugation-ready particles with either surface-bound carboxyl or amine groups. *In vivo* use contemplated see caveats on nanoparticles, however. Quantum Dot now offers their "Qdot nanocrystals" ranging from 525-655 nm conjugated to streptavidin, biotin, or Protein A. Qdots conjugated to specific secondary antibodies now exist. "Crystal Light" is a semiconductor nanocrystal made by researchers at Los Alamos National Laboratories that can amplify light. It has the cadmium-sulfide core and a zinc selenide shell. Electrons get trapped in the core while positively charged holes move to the shell, keeping the particles from interacting with each other and annihilating each other – this lets the nanocrystal amplify light, a crucial requirement in making a nanocrystal laser. They probably will be built into optical communication fibers or into lab-on-a-chip devices (See also Image Analysis section). Invitrogen also has Qdot nanocrystal fluorescence for mycobacterail culture (Becton-Dickinson). It is based on oxygen consumption. They also have BACTEC systems, which use an increase in fluorescence proportional to CO_2 presence indicating viability of the tested culture.

J. Nuclear Magnetic Resonance

Instruments involve a magnet (e.g. superconducting) probe, consol, and computer. Pulsed field NMR. Used for structural determination of complex molecules and protein-ligand interactions. Nuclei of ^{1}H. ^{31}Phosphorus, ^{19}Fluorine and ^{15}N, can be used for metabolic studies, useful in drug discovery, development and proteomics and genomics research. Instrumentation sensitive to the spin of resonance of the molecules-fingerprinting, ID. Varian Co., Abbott Laboratories, NMR spectroscopy has been used for metabolic analyses of biofluids (see "Metabolomics").

K. Radio-labeled Compounds

Measurement of metabolism and metabolic end products during growth, radio-labeled immunoglobins. Radio-labeled probe for DNA hybridization, ^{14}C, ^{125}I, ^{3}H, ^{35}S. Automated Geiger and gamma counters. BACTEC 460 monitors $^{14}CO_2$ production from radio-labeled substrates. (e.g. ^{14}C sugars, ^{14}C amino acids, etc. now mostly used for mycobacterial cultures). Binax Co., Packard Instrume. · Co. Fewer use now because of radiation restrictions on purchase disbursements and ultimate disposal. However, stable isotope labeling is being used to label amino acids (Thermo Scientific Pierce SILAC kits).

L. Infra-Red Detection

CO_2 production monitored by absorption in infra-red range. Detection of microorganisms, sterility, testing, etc. BACTEC NR660 (Becton Dickinson). IR and Near IR cameras (Indigo Systems). Wilks Enterprise Inc. offers two portable mid-IR analyzers for biodiesel, free fatty acids and water in feed stocks.

M. Electrical Measurements

1. Impedance

   Resistance of a circuit element to the flow of an alternating current through a conducting material. Voltage/current (E/I). Metabolic changes result in changes in impedance. Sterility testing, microbial metabolism, inhibition/growth studies/clinical application Bactometer (BioMerieux Vitek, Inc.) M 64, M128 Malthus (Automated) and RABIT (Rapid Automated Bacterial Impedance Technique)

2. Electrode Probes

   Use of pH meter electrodes to detect electrical changes in reactions involving growth, enzymes or immunological reactions. Gas, pH, and ion-sensitive electrodes (Corning Orion) detect presence of important compounds (ammonia, amino groups, nitrates, oxygen, and sulfides). Fermentation monitoring, detection of microorganisms in water or urine.

N. DNA/RNA

DNA probes, hybridization, polymerase chain reaction (PCR). Many are enzyme-linked at the final steps or need concentration/purification by electrophoresis chromatography, etc. Eastman Kodak testing rapid PCR instrumentation for several bacteria (not on market yet).

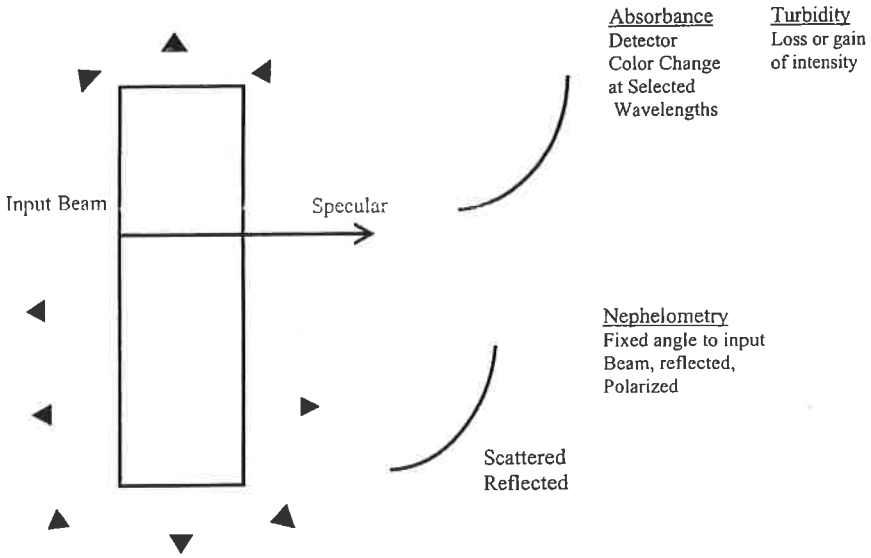
O. X-Ray Chrystallography

Identification of compounds by their crystal 3-D structure and bonding. Bruker AXS markets the SMART X2S, an automated benchtop X-Ray diffractometer including data collection, processing and structure solution.

III. BIOCHEMICAL DETERMINATIONS (See also V Biosensor Systems)

A. Photometry

Optical properties of a medium characterized by refractive index (RI) if uniform, light passes through undeflected. When RI varies, e.g. particles (microorganisms) present, part of radiation is scattered. Also, changes in pH sensitive dyes recorded.



1. BacT/Alert-3D BioMerieux growth, pH, changes when CO_2 across a semi-permeable membrane into dye area. Results in a color change from green to yellow. Automated incubator/reader, barcode wand included 240 bottles. AST/, and /LYM for high acid food and beverage, FAM

2. BD Pheonix, Identification and Antimicrobal Susceptibility Testing System (ID/AST) Fluorogenic and chromogenic substrates for ID oxidation/reduction and turbidity for susceptibility testing (AST).

3. Biolog (Biolog, Inc. Hayward, CA). 96 well plate-specular portion of white light passing through selected chemicals, color change of tetrazolium dyes recorded. Turbidometer for inoculum an adjustment, plate reader adding yeast now into microarrays. Data base and increasing lactobacilli database to over 1,000 organisms.

4. MicroScan and Walk Away Plus 40, 51 and 96 (Siemans Healthcare Diagnostics USA) has a stacking carousel ("tower") for 40 or 96 panels for biochemicals and MIC dried panels). Based on absorbance also has a rapid 2 hour ID based on fluorescence of 7 - amido-4 methylcoumarin or 4-methyl umbelliferyl compounds. Barcode and biochemicals on panel tells instrument which reader/wavelengths to use.

5. Mini-API (BioMerieux Vitek, Inc. /API) specular beam identical reagents to manual cupule system. Autoinoculator, autoreader, absorbance (color changes with growth on biochemicals) reads the API strip (used in Europe).

6. BD BACTEC MGIT 960 for Tuberculosis (fluorometric anaylysis)

7. Vitek (BioMerieux Vitek, Inc.) formerly called "AMS", specular portion of white light beam passing through selected growth media, dyes and growth changes, absorbance and turbidity, various "cards".

8. Robotic Systems (Zymate) Assays for *Limulus* amebocyte lysate assay (LAL) chromogenic substrate. Infinite other possibilities.

9. Nanodrop ND 8008, sample spectrophotometer (1.0 ml samples).

10. Dynamic Light Scattering: Capable of measuring a large population of particles in a very short time with no manipulation of dispersing medium. The "Zetasizer Nano" is a bench top instrument combining dynamic static and electophoretic light scattering measurements. It can be combined with size exclusion chromatography in Malvern Instrument's "Zetasizer Nano."

11. Static Light Scattering can be used to characterize protein-protein interactions as well as reversible hetero-association Calypso SP3 instrument (Wyatt Technology Corp.) Also their automated DynaPro Titan Plate Reader for biomolecular characterization.

IV. IMMUNOLOGIC IDENTIFICATION SYSTEMS (See also V, biosensor Systems)

A. Photometry EIA

1. Biomeck 1000 Automated Laboratory Workstation (Beckman) Non-dedicated. Tubes, 96 well microtiter plates, collimated beam through well bottoms, ELISA tests e.g. *Brucella* antibodies, DNA sequencing, automated.
2. SYLVA Systems (San Jose, CA)
   a. E. T.S. Dedicated instrument for drugs of abuse in serum and urine.
   b. XL System for *Chlamydia*
   c. Sell kits and reagents for drugs of abuse that can be used on general chemistry analyzers ("non-dedicated")
3. Microtiter Plate Readers for individual tests: Dynatec, Biorad, Fisher, ARTEK etc. Many others! Some very fancy with plate stacking, shakers, incubators, some for fluorescence, etc.

B. Fluorescence

1. TDX (Instrument) (Abott) Fluorescent polarization immunoassay (FPIA) at present; drugs of abuse, therapeutic drugs, dedicated kits.
2. INIX Instrument (Abbott) reads two types of tests
   a. MEIA: microparticles are the solid matrix. Dedicated immunoassay kit – for toxoplasma, IgG, IgM, CMV, Hepatitis viruses (core, IgG and IgM to Hepatitis A, core Hepatitis B antigen, etc.)
   b. FTIA: to date only for T 4 uptake, theophylline, in future, other tests. Stand alone microtiter plate reader could also be used with these kits.
3. Vidas (BioMerieux Vitek, Inc.)

   Antigen and/or antibody detection mycoplasma, mycobacteria, HIV antigen, Torch Panel, based on fluorometric end points.

C. Radionuclides (RIA)

1. Genesis gamma counter for $^{125}1$, ^{57}Co (Ciba-Corning) non-dedicated.
2. Kinet Count (Binax Corp. S. Portland, Maine) formerly a Vitek instrument, now Binax providing reagents (40 well RIA) each well surrounded by a detector.

D. Chromatography (See Also Photometry and Particle Counting)

1. Stratus Immunoassay System (dedicated) EIA using radial partition chromatography mainly antibiotics, drugs, etc., in serum. Fluorescent end point with final wash. Newest instrument in series the Stratus Intellect, computerized (uses "Windows" software). Thermo Scientific Pierce Chromatography Cartridges (with Protein A, G for antibody purification).

E. Luminescence

Solid phase immunoassay bioluminescent or chemiluminescent compounds conjugated to IgG or antigens (CELISA,LIA) horseradish peroxidase as catalyst. Luminal or Arylacridinium esters (Ciba-Corning, Nowood, Mass., Magic Lit Systems, kits). Luminescent immunoassays (LIA, CLIA) have semi-automated readers available for microtiter plates (Dynatech). Luminometers also available. E/LUMINA 2-E (automated, "glow" and "flash") (Source Sci. Syst. Garden Grove, CA). (See previous bioluminescence, chemiluminescence, etc.)

F. Miscellaneous

Enzyme- immunoassays performed on microscope slides or nitrocellulose paper can be read by Image Analysis systems, densitometers, and eyeballs.

V. BIOSENSOR SYSTEMS

Definition: A biosensor is a special biorecognition entity ("sensor") in close proximity or linked to a signal receptor system ("transducer"). The transducer records and changes in intensity related to concentration and/or activity that occur when the sensor reacts with its target ("the analyte"). Sensors can be very specific molecules: enzymes, antibodies or antigens, DNA or RNA molecules, neuro-ganglions. There are also non-specific sensors: microorganisms, tissues cultures, cells, etc., (depending on the desired type of reaction). The transducting portion may be based on electrochemical/electric, optional, thermal, etc. Most instruments can be computer—driving/recorded. **Note:** Combinations of sensor and transducer are multiple.

1. **Electrochemical/electric**
   a. Potentiometric: voltage changes on a pH or other electrode antibody-antigen reaction at constant current. DNA detected in pharmaceuticals. (Threshold Systems Instrument Molecular Devices, Menlo Park, CA), drugs, whole cells.
   b. Amperometric: current changes are measured at constant voltage. Enzyme activity oxidase (Biocheck Instrument) freshness of meat and fish, Fluorescence).
   c. Piezoelectric
   d. "Optrodes": electrodes/optical/fiber combination usually for glucose analyses.
   e. Optics (in general): Very adaptive to combination with amperometic, piezoelectric, luminescent, and many other transducer combinations. Enzymes, antibiotics, antibody-antigen, whole cell activities, flow-through "real time" assessments. Very little use of radio nuclides, much use of turbidity (e.g. Cadmium toxicity effect on protozoal growth fluorescence and absorbance dyes, miscellaneous compounds automated instrument (E.Lilly, Indianapolis) with 18 biosensors. "LAPS" Cytosensor (Molecular Devices, Menlo Park, CA). Optical diffraction occurs when antigens bind to alternating rows of antibodies on a disposable silicon chip A CD-type He Neon laser beam hitting the chips diffracted in Ag-Ab reaction has occurred (Idetek Inc., Sunnyvale, CA). Static Light Scattering (SLS) has been used to characterize protein-protein interactions.
   f. Surface Plasmon Resonance (SPR): BIACORE (GE Health Care) also BiAlite Upgrade Kit. SensiQ Pioneer automated, semi-automated, and manual SPR systems. Changes in refracted angle of polarized light projected through a half circular prism when a reaction occurring on a thin layer of gold or silver is hit by the beam. Antibody-antigen, DNA-DNA hybridizations, DNA synthesis, protein, protein, aptamer reactions. Bio-Rad's Automated ProteOn XPR36 Protein Interaction Array System is a 6 x 6 multichannel SPR platform. Biocore also produces several systems that rely on fundamental flow cell-based fluidic device to clean and purify samples before SPR analysis. SPREETA (Texas Instruments sold to Sensata Technologies), a hand held SPR sensor-salmonellae.

2. **Miscellaneous:** Thermistors, calorimeters, mass spectrometers. Matrix assisted laser desorption/ionization "MALDI" time-of-flight mass spectrometry-immunoassay "MSIA". Infra-red glucose sensors, heavy metal ion detection, antibody-antigen reactions, enzyme reactions. Pressure sensitive devices

3. **Caveats**

   A. Stability, shelf life

   B. Reproducibility

   C. Ease of Reconstruction of sensor

   D. Non-specific binding to sensor/transducer

   E. Disposable sensors

   Very bright once above caveats are settled. New types of sensors; transducers and combinations will change the detection and characterization methodology we presently use.

Microarrays and Microfluidics

1. **Labs on chip**

   Microfluidics is the art of precisely controlling microliter volumes of fluids; evolved with serious potential by companies providing instruments using "chips" for many biological applications such as PCR, protein crystallization and bio-reactors. Digital photography with chips and pixels (Leica Microsystems). Gyros AB has released the Gyrolab Biaffy, a CD-based proteomics microlaboratory. This lab on a CD type platter uses sandwich immunoassays for protein quantification. La Clair has developed a data CD using bytes to represent ligands, then printed the molecules, added samples, washed and played. Caliper Life Sciences has several chip platforms (one, planar chip technology, was licensed to Agilent for their 2100 Bioanalyzer and to BioRad for use with the Experion automated electrophoresis system. Shimadzu's "Multina" is a fully automated microchip (reusable) Electrophoresis System for DNA/RNA Analysis, e.g. identification of 5 meat products. (BioTechniques 46: (3) 2009.)

2. **Microfabrication**

   NUNC and Schleicher and Schuell (Whatman) have microarray slides. MT laboratory is developing a "Nanoruler" which is 10 to 100x faster in patterning parallel lines and spaces across surfaces larger than 200 mm in diameter. Can make lines only a few hundred billionths of a meter apart with one nanometer precision. Used in gratings, chip production, etc.

3. **Microfabrication of silicon chips for PCR reactors and electrophoresis**

   Biotrove's "Open Array" technology performs thousands of nanoliter-volume PCRS. Chips for DNA hybridizations and for HPLC micro-sized. Sigma has new guide: "Proteomics and Protein Expression" (free). See also the following articles.

4. Uses

   Microfluid mixers using bubbles as mixers. Laser valves to control reactions in chips are becoming more frequent (SpinX Technologies). Environmental monitoring, cell sorting, separation techniques, biological warfare detection, DNA sequencing, drug discovery. Biolog Phenotype Microarray's Omnilog, GENIII technology can quantitatively monitor and measure thousands of cellular phenotypes all at once (microbial id/characterization). Roche has a NimbleGen microarray scanner.

5. **Single-nucleotide polymorphisms ("SNP's")**

   Single-based primer extension technology has been automated by Beckman Coulter: Genome Lab SNP stream Genotyping Systems (4600-800,000 genotypes analyzed per day). Multiplexed systems. **THE FUTURE OF MICROFLUIDICS WILL INVOLVE CELL-BASED ASSAYS AND APPLICATIONS. FOR EXAMPLE, CULTURE OF ANIMAL AND HUMAN CELLS AND GENERATION OF RESPONSE CURVES FOR SIGNLE CELLS EXPOSED TO TREATMENT.**

What is a Nanoparticle?

A definition: There is no accepted international definition of a nanoparticle, but one given in the new PAS, 71 document developed in the UK is: "A particle having one or more dimensions of the order of 100 nm or less".

There is a note associated with this definition: "Novel properties that differentiate **nanoparticles** from the **bulk material** typically develop at a critical length scale of under 100nm."

What is different about a nanoparticle?

There is no strict dividing line between nanoparticles and non-nanoparticles. The size at which materials display different properties to the bulk material is material dependant and can certainly be claimed for many materials much larger in size than 100nm.

Definitions certainly become more difficult for materials that are a very long way from being a sphere, such as carbon nanotubes, for example. One of the aims for these materials is to grow them into long tubes, certainly not 'nano' in length, but as they have a diameter in the order of 3nm for a single walled tube, they have properties that distinguish them from other allotropes of carbon, and hence can be described as 'nanomaterials'.

Manufacturing methods for nanoparticles

Many of these nanomaterials are made directly as dry powders, and it is a common myth that these powders will stay in the same state when stored. In fact, they will rapidly aggregate through a solid bridging mechanism in as little as a few seconds. Whether these aggregates are detrimental will depend entirely on the application of the nanomaterial.

If the nanoparticles need to be kept separate, then they must be prepared and stored in a liquid medium designed to facilitate sufficient interparticle repulsion forces to prevent aggregation.

Manufacturing nanoparticles

There are four fundamental routes to making nano materials.

- **Form in place**

  These techniques incorporate lithography, vacuum coating, and spray coating.

- **Mechanical**

  This is a 'top-down' method that reduces the size of particles by attrition, for example, ball milling or planetary grinding.

- **Gas phase synthesis**

  These include plasma vaporization, chemical vapor synthesis and laser ablation.

- **Wet chemistry**

This is the range of techniques that are most applicable for characterization by light scattering techniques. These are fundamentally 'bottom-up' techniques, i.e. they start with ions or molecules and build these up into larger structures.

Nanotechnology

Nanotechnology refers to the building of small machinery or compounds on a nanometer scale (billionth of a meter) using many of the principles of macroscopic engineering. Objects are built atom by atom, bearings, axles of diamond-like lattices of carbon, pumps and tiny computers. The goal is precision and control at the level of individual atoms. A comparison with biologic nanotechnology and its organic and flexible forms is necessary to understand this new and growing field. These include nucleic acid molecules, proteins and other molecular machinery. Enclosing dye particles inside of nanoparticles enhances 1000x sensitivity of reactions (Seydak, M. Biosens Bioc 20: 2454 (2005)). Use of liposomes containing dyes also effective, can be coupled to antibodies, as well as magnetic nanoparticles conjugated to dye molecules and antibodies.

A word of caution of possible unforeseen effects of inhalation or ingestion of these small sized particles. Impacts on health and environment? Possible restrictions on use (compare to asbestos fibers).

Quantum dots (QD) are colloidal nanocrystalline semiconductors having diameters between 1nm and a few microns, which, upon broad band irradiation, emit light at certain wavelengths that are directly related to their size. For example, Cdse Qdots at 2nm emit green light (550nm) while 4nm dots emit red radiation at (630nm) very stable. Invitrogen carries antibody conjugates of Qdot 625 nanocrystals.

Fluorescent labeling of nanotubes with Q dots; single walled nanotubes for nanophotonics; quantum computing and probes for research.

Carbon nanotubes have been "hidden" under a coat of glycans, which can mimic various cell recognition functions and can result in highly specific cell targeting depending on the "coating materials" (Chen et al, JACS, April 2006). Nanometer-sized electrochemical probes can penetrate mammalian cell membranes without causing damage (Sun, P. PNAS USA 105. 443-448 (2008).

Metabolomics and Bioinformatics

Along with genomics and proteomics, metabolomics is the next step: the whole metabolic profile of the cell and the understanding of cellular functions. Metabolomics should provide complementary data sets which, together with genomics and proteomics, should be used to construct computer network models to describe cellular functions. This data should be of help with the development of new tests and the development of tests for regulatory validations. Microarray data is invaluable here. This would include data from controls and tests.

Biolog Microarrays (Biolog, Inc., Hayward, CA) new Phenotype array technology Omnilog system (2000 tests) uses GENIII technology.

CONCLUSIONS

A. Robotics are here to stay.

B. Automated Instruments are expensive.

C. Errors may occur (possibly less than with humans).

D. Back-up kits, materials and quality control measures are necessary.

E. Skilled data interpretation is still needed.

F. Space constraints, work load, company reputation, ease of upgrading to new technologies, costs, etc., all must contribute to purchase decision.

Look in the future for more references to small duplex dsRNA cell RNAi or siRNAs for gene silencing via interference and to assess gene function.

Nanotechnology Now

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