THE FUTURE OF POINT OF CARE TESTING

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I have nothing to disclose.
LEARNING OBJECTIVES

- Review how far point of care testing has come.
- Discuss up and coming tests and instruments
- Contemplate what POCT will look like in the next decade.
THE DOCTOR’S BAG FOR THE NEW MILLENIUM

- Article in the Well Section of the New York Times in October 2012
- Written by Abraham Verghese, MD
- He discusses how the medical bag his uncle carried as a physician in India in the 1970s, which was more of a “mobile office” has been replaced
- Statement he makes at the end of the article relates not just to physicians, but also to point of care testing...
The first diagnostic test is thought to have been urine testing for diabetes.

- Healers in 1500 BC noticed that ants were drawn to the urine of patients who had a mysterious emaciating disease.
- In the 1600s, tasting the urine was the method to diagnose diabetes.
  - Sweet tasting urine = diabetes.
The earliest and most basic POC test was dipstick urinalysis

- Urine dipstick, developed in 1957, was the first true POC device
  - Made use of advances in technology from the pharmaceutical industry for testing urine for albumin, occult blood and acetone using dry reagent tablets
  - Borrowed technology from the newspaper industry to create reagent impregnated papers
  - Other tests were added to the dipsticks, including total protein, ketones, nitrite, specific gravity and leucocytes
HISTORICAL VIEW OF POCT

- Next technological breakthrough
  + Development of the handheld glucose meter
  + First blood glucose meter was the Ames Reflectance Meter (A.R.M) in 1969
    - Only available to physicians
    - Components were Dextrostix, a reflectance meter and a photoelectric cell
  + First meters developed for home use came on the market in the early 1980s
    - Ames Glucometer
    - Accu-check meter by Boehringer Mannheim (now Roche)
    - GlucoScan by LifeScan (now Johnson and Johnson)

www.cap.org
http://www.mendosa.com/memories.htm
Clinical Laboratory Improvement Amendments of 1988

- Began as the Clinical Laboratory Improvement Act of 1967
  - Original law only covered laboratories doing business across state lines, and many amendments were added to regulate all laboratories performing tests on human specimens
- In 1987, articles in newspapers and magazines were published and television programs aired concerning the quality of laboratory testing
  - Specifically concerning validity of cholesterol screening and accuracy of Pap smears
- Congress held hearings in 1988 and heard from victims of faulty laboratory testing
- Enacted to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed
  - Defines a laboratory as any facility that performs laboratory testing on specimens derived from humans for the purpose of providing information for the diagnosis, prevention and treatment of disease or for assessment of health.
  - This definition was expanded to include any site where clinical laboratory testing occurs
    - This includes sites where POCT is performed, such as the patient’s beside, physician operated laboratory or clinic

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Passage of CLIA88 led to more regulations, including the introduction of the complexity model for test methods

- List of original waived tests
  - Urine dipstick or tablet, fecal occult blood, ovulation, urine pregnancy, ESR, hemoglobin, blood glucose, microhematocrit
  - List now includes nearly 100 waived analytes with over 1000 waived test systems

- Searchable database of all FDA approved tests with testing category can be found on the FDA’s website

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EVOLUTION OF POCT

- Initially, most POCT were used in physician offices or in the home
  - 3 tests comprise the bulk of POCT in the US
    - Urinalysis by dipstick
    - Blood glucose
    - Urine pregnancy
- In the early 1990s, handheld glucose meters used in the home made their way into hospitals
  - Ability to manage blood glucose of a diabetic patient at the bedside provided a level of convenience that could not be matched by the central laboratory
  - Bedside monitoring became the standard of care in the majority of hospitals
- Central laboratories in hospitals considered the testing supplemental
  - Considered the testing substandard
  - Competitor for their services
  - Neither nursing or the laboratory wanted to assume responsibility for management of the testing

www.cap.org
Clinics in Laboratory Testing: Point-of-Care Testing, Lewandrowski, 2009
This attitude slowly changed as the number and type of devices grew

Growth has been stimulated by:

- Technological advancement
- Demands for faster turnaround times
- Waived testing designation under CLIA88
- Creation of specialty clinics
- Demand for self-testing and patient control

Evolution is continuing...
NEW AND UP AND COMING TESTS
WHERE IS POCT GOING?

- Much of the research and development is looking toward bringing testing to underserved populations and developing nations.
- Even in patients who are high risk, testing is limited by several factors:
  - Expense
  - Location
    - Most testing takes place in centralized or regional laboratories.
WHO has developed guidelines for development of diagnostics for low-resource settings

ASSURED

- Affordable
- Sensitive
- Specific
- User-friendly
- Rapid and Robust
- Equipment-free
- Deliverable to end-users
DETECTION OF MALARIA

- Widespread across the developing world
  - 200-500 million illnesses each year
  - 1 million deaths each year
- Over-prescription of inexpensive drugs has led to drug resistance
  - Effective drugs are much more expensive, so accurate diagnosis is key
- Nucleic Acid Tests (NATs) have been suggested as fulfilling the criteria
  - Can detect extremely low level infections with high sensitivity and specificity
  - Proposed methods need to be tested in the field

Traditional POC paper-based devices (i.e. lateral flow devices) can run more than one test in a series
- Require that the tests be compatible with each other in terms of buffers and reagents
- No cross reactivity between the assays

Paper-based microfluidic devices consist of hydrophilic paper channels defined by patterning of hydrophobic barriers or by cutting the material.
- Directs fluid towards specific detection zones and in 3 dimensions
- Performs operations such as mixing, splitting and filtration automatically
- Allows multiplexing of a small sample volume (<40 μl) in independent assays done in parallel with no worry about cross-reactivity

Assays are portable and disposable
Proof-of-principle studies have tested the ability to conduct clinical chemistry, enzymatic, immunoassay and enzyme-linked immunosorbent assays
Minimal validation with clinical specimens
Monitoring liver function is standard of care for patients with liver disease or those taking medications with hepatotoxicity

- Particularly patients on standard therapy for tuberculosis and/or HIV
- Guidelines call for baseline and serial monitoring of AST and ALT for those on therapy

Development of a paper-based, POC assay for rapid, semi quantitative measurement of AST and ALT from a finger stick whole blood specimen
Two layers of patterned paper

Plasma separation membrane
  + Blood applied to back of device
  + Red and white blood cells filtered out
  + Plasma wicks into 5 detection zones by hydrophobic channels

Laminated cover of polyester film

Results read after 15 minutes
Visual color readout is sorted into “bins” of <3x ULN, 3-5X ULN, and >5x ULN, which corresponds to cut offs used in clinical management decisions.
ANALYTICAL PERFORMANCE

- Overall accuracy for device was >90%
  + Placing patients in the correct “bin”
- Clinically acceptable agreement with the paper test and the gold standard automated assay method
  + ALT underestimated by 9% on average
  + AST overestimated by 12% on average
- Device stability only tested out to 11 weeks
PROGRAMMABLE BIO-NANOCHIP (P-BNC) TECHNOLOGY

- Provide the ability to rapidly secure sensitive, reliable simultaneous measurements of key biomarkers at the POC

Programmability
  + Sensor can function as a standard platform that can serve multiple applications by inserting a molecular level “code”
    - Biomarker-specific reagents

Bio
  + Capacity to measure and extract the bio-signatures associated with disease progression

Nano
  + Capacity to miniaturize the system through use of nano-nets for capture and quantum dots for increased signal generation

Chip
  + Capacity to mass-produce sensor elements similar to microchips, that leads to high performance at low cost
P-BNC SYSTEM

- Portable self-contained analyzer
- Single use, disposable lab card
- Chemical processing unit is an array of beads
- Fluorophores are throughout the beads
- Analyte sandwiched on nanometer agarose strands
BIO-NANOCHIPS FOR CVD DIAGNOSIS

- Use of saliva to identify biomarkers of AMI
- Measured levels of 21 proteins in AMI patients and healthy controls
  - Narrowed down the top 10 most informative biomarkers
  - Determined the area under the curve (AUC) for each biomarker and combination of biomarkers
    - C-reactive protein and myoglobin together had an AUC = 0.94, which was superior to that of ECG alone (AUC = 0.6)
    - Created a duplex test on the P-BNC platform
      - Results correlate well with a clinical reference analyzer

Christodoulides et al. Methodist Debakey Cardiovasc J. 2012 Jan;8(1):6-12
Biosensors at the point of care

Biosensors consist of biorecognition elements specific to the analyte of interest and a physiochemical transducer to relay the resultant signal from the biorecognition event.

- Integrated blood barcode chip
  - On-chip plasma separation from whole blood and rapid in situ measurement of multiple plasma proteins
    - Can detect hCG over a $10^5$ concentration range
- Antibody microarrays inside microfluidic devices
  - Immunophenotyping of leukocytes from whole blood

MINIATURIZED TECHNOLOGY

- Taking current technology and shrinking it down allows more tests to be placed on a single device and allows more testing with a smaller sample volume
  - Microarrays (multiple microscopic detection spots placed on a chip)
    - Determination of inflammatory and sepsis markers CRP, IL-6 and PCT in plasma
    - Simultaneous analysis of 12 plasma proteins (PSA and 11 cytokines) in 10 minutes from a single drop of blood
    - Electrochemical immunosensor to simultaneously detect tumor markers (CA 153, Ca 125, Ca 199 and CEA) with immobilized gold nanoparticles with peroxidase-tagged antibodies

Olasagasti and Ruiz de Gordoa, Translational Research 2012;160:332-345
Aptamers

- In vitro created nucleic acid molecules that are capable of binding specific target molecules or cells
- Can be used for analysis of protein samples
  - Used in a dry-reagent strip application for the detection of thrombin
  - Detection of Ramos cancer cells (Burkitt’s lymphoma cell line)
    - Can be used even when certain structural knowledge of the target is unknown

Magnetic particles

- Convenient way of separating target proteins
- Detect proteins based on their magnetic properties
  - Analysis of CRP could be done with 4 μl of whole blood within 5 minutes and had good correlation with reference method

Olasagasti and Ruiz de Gordo, Translational Research 2012;160:332-345
Changes that we will see in the future do not only include new tests, instruments or technologies....

It will also include using the current technology in new ways.
SMART PHONES AND DIABETES

- iBG Star
- iPhone and iPad compatible glucometer
- Can sync data with a diabetes manager application
- Data can be emailed or shown to the patient’s healthcare provider

- Glooko
- Created a Logbook app for iPhone
- Cable connects iPhone to glucose meter (supports 17 different meters) and allows download of meter data
- Can email or fax data to healthcare provider
ORAQUICK IN HOME TEST FOR HIV

- Approved by the FDA for home use in July 2012
- Same test as the OraQuick Advance that has been in use by health professionals since 2004
Stroke is one of the main causes of death worldwide and a common cause of disability. 90% of strokes are due to cerebral ischemia, 10% due to cerebral hemorrhage. Approved treatment of ischemic stroke is recanalization of occluded arteries by thrombolysis within the first hours of symptom onset. Difficult to implement due to need for laboratory testing to rule out contraindications to thrombolysis. 15-40% of patients arrive at the hospital in time to receive thrombolytic treatment, and only 2-5% of patients actually receive the treatment. Of those who do, outcome is closely related to time of treatment. Can stroke treatment be brought to the patient? Will it improve outcomes?
WHAT IS A MOBILE STROKE UNIT?

- Team
  - Paramedic
  - Stroke physician
  - Neuroradiologist

- Equipment
  - CT scanner
  - Telemedicine system
  - POC laboratory system
    - PocH 100i (platelet count, leukocyte count, erythrocyte count, hemoglobin and hematocrit)
    - Hemochron Jr (INR and APTT)
    - Reflotron plus (GGT, amylase, glucose)

- Patients in the control group also received point-of-care laboratory testing instead of testing from the central laboratory
Patients treated with the MSU were given treatment twice as fast as those in the control group.

Very critical given the accepted concept that “time is brain”
**DID FASTER TREATMENT LEAD TO BETTER OUTCOMES?**

- No significant difference in outcomes between the two groups.
- Possible reasons:
  - Low power of study
  - Only enrolled 28% of patients screened
  - Did not assess cost

### Table 3: Prespecified safety endpoints and other serious adverse events

<table>
<thead>
<tr>
<th></th>
<th>MSU group (n=53)</th>
<th>Control group (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prespecified safety endpoints</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival at day 7</td>
<td>47 (89%)</td>
<td>45 (96%)</td>
</tr>
<tr>
<td>Stroke-related or neurological death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatal ischaemic stroke</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Fatal reinfarction</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Fatal primary ICH</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Fatal secondary ICH</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Non-fatal secondary ICH (change in NIHSS ≥4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-fatal cerebral herniation and symptomatic oedema (change in NIHSS ≥4)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Peripheral haemorrhage†</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Other serious adverse events‡</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>46 (87%)</td>
<td>37 (79%)</td>
</tr>
<tr>
<td>Non-fatal reinfarction</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Secondary ICH (change in NIHSS &lt;4)</td>
<td>0</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Myocardial infarction‡</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2 (4%)</td>
<td>6 (13%)</td>
</tr>
<tr>
<td>Other infection</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Data are n (%). MSU = mobile stroke unit. ICH = intracranial haemorrhage. NIHSS = National Institutes of Health Stroke Scale. †ICH was a subarachnoid haemorrhage secondary to thrombolysis. ‡Haemorrhage was associated with rupture of an unknown aneurysm of the lenticulostriate artery one day after intravenous alteplase. †One patient in the control group concomitantly had ICH and pneumonia, one patient in the MSU group concomitantly had myocardial infarction, recurrent stroke, and pneumonia. §One patient in the MSU group with myocardial infarction died at day 2.
Enumeration of CD4 lymphocytes is an essential diagnostic tool in HIV infected individuals for initiating therapy and monitoring its efficacy.

POC CD4 testing can improve access to treatment by reducing patient-loss-to-follow up.

**PointCare NOW CD4 system**
- Fully automated closed system that requires no sample preparation
- Impedance orifice technique to count WBC
- LED multi-angle light scattering for four part differential
- Blood is mixed with anti-CD4 antibody-coated colloidal gold particles to give them a unique refraction signature
- Absolute CD4 counts are calculated from WBC, lymphocyte % and CD4% results

Significantly overestimated CD4 absolute counts with a mean relative bias of 35% when compared to reference method

Performance of the instrument at this time is inadequate for HIV clinical management

Bergeron et al PLOS ONE, 7(8):e411166
Exciting innovations in technologies for detection of nucleic acids as well as sequencing and interpretation are being published daily.

- While not quite ready for the point of care, the $1000 genome is closer than ever to becoming a reality.
- Amplification-free nucleic acid testing technologies are in development, which will be very useful in POC settings.
  - Bacterial and viral infectious agents
  - Diagnosis of specific cancers
**ANALYSIS OF NUCLEIC ACIDS**

- **Main challenge is the small amount of sample**
  - Amount of sample or signal intensity must be increased, while keeping the noise low
  - **Signal amplification**
    - Detection of IL-8 mRNA in saliva- proposed biomarker for oral cancer
      - Hairpin RNA coupled to horseradish peroxidase (HRP) complementary to IL-8 mRNA
      - In absence of IL-8 mRNA, the hairpin was self-annealed and HRP could not interact with it’s substrate
      - In the presence of IL-8, hairpin was able to hybridize, releasing HRP to interact with substrate to produce a measureable electrochemical signal

Olasagasti and Ruiz de Gordoia, Translational Research 2012;160:332-345
ANALYSIS OF NUCLEIC ACIDS

- Sample amplification
  - Traditionally requires a thermocycler for polymerase chain reaction (PCR)
  - Methods to amplify DNA without a thermocycler are being explored
    - Loop-mediated isothermal amplification (LAMP)
      - Technique relies on strand displacement by DNA polymerase and primers that can amplify the target at a constant temperature
        - Prototype device was low cost and disposable
        - Could analyze swab-collected samples in around 90 minutes
        - Detected 17 copies of MRSA genomic DNA and foot-and-mouth-disease virus from 100 μl of epithelial homogenate
        - Readout is a simple colorimetric detection system
    - Other uses of this technology
      - Detection of H1N1 seasonal influenza virus within 30 minutes from 2 μl of clinical sample
  - Helicase dependent amplification
    - Strand displacement with a thermophilic helicase, labeled primers were used for amplification and detection
      - Genomic DNA from *N. gonorrhoeae* and *S. aureus*
      - Has not been trialed on clinical samples

Olasagasti and Ruiz de Gordoa, Translational Research 2012;160:332-345
POINT-OF-CARE GENETIC TESTING

Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial

Jason D Roberts, George A Wells, Michel R Le May, Marino Labinaz, Chris Glover, Michael Froeschl, Alexander Dick, Jean-Francois Marquis, Edward O’Brien, Sandro Goncalves, Irena Druce, Alexandre Stewart, Michael H Gollob, Derek Y F So  

Lancet 2012; 379: 1705-11

- First proof-of-concept study showing the feasibility of point-of-care genetic testing in clinical practice.
- RAPID GENE trial
  + Prospective, randomized blinded study to assess CYP2C19*2 genotyping to improve platelet suppression in patients undergoing coronary artery stenting.
Sequence variants of CYP2C19 are responsible for a substantial portion of platelet responsiveness after administration of clopidogrel (Plavix)

- CYP2C19*2 is a loss-of-function allele
- Heterozygote carriers have a threefold higher risk of stent thrombosis compared to individuals with the wild-type allele, and homozygous carries have an even higher risk

200 patients undergoing percutaneous coronary intervention (PCI) were randomly allocated to either rapid POC genotyping or standard treatment

- Genotype-guided strategy involved use of prasugrel instead of clopidogrel for patients who were carriers
- Primary endpoint examined was high platelet reactivity, which is associated with increased adverse cardiovascular events

RAPID GENE

- Spartan RX CYP2C19 device
  - Four steps intended to be done in less than 8 minutes
    - Acquisition of a buccal swab
    - Insertion of the swab into an assay cartridge
    - Insertion of the reaction solution into the testing device
    - Push a button to begin the analysis
      - Results were available within 60 minutes
      - Wild-type (*1/*1), heterozygous (*1/*2), homozygous (*2/*2)
  - Operators were clinical trial nurses who received 30 minutes of training, and had no previous laboratory experience
  - Genotypes of all patients were confirmed by conventional DNA sequencing

187 patients had complete follow up
+ 91 in rapid genotyping group
  × 23 patients (25%) had at least one copy of *2
  × 4 (4%) were homozygous
+ 96 in standard treatment group
  × 23 (24%) patients had at least one copy of *2
  × 3 (3%) were homozygous
+ 1 patient in the rapid genotyping group was incorrectly identified as (*1/*2)
  × Sensitivity 100%
  × Specificity 99.3%
PRU > 234 predicts an increased rate of major adverse cardiovascular events, but some studies cite 208 as a better cut off

Significant reduction in the rate of high platelet reactivity in CYP2C19*2 patients who had rapid genotyping compared to those who received standard treatment
WHERE DO WE GO FROM HERE?

http://www.sacredart-murals.co.uk/mural-gallery/toy-story-mural-shrek-monsters-inc.htm
One of the fastest growing aspects of clinical laboratory testing
- Estimated to be increasing at least 10-12% per year, with some areas increasing 30% per year

In contrast, central laboratory testing has grown 6-7% per year

What is driving this increase?
- Technical advances making POC testing more accurate, more robust, cheaper and easier
- Changes in the clinical environment that makes it necessary for shorter hospital stays and quicker patient turnaround
- New therapies that require rapid laboratory results in hospitals but also in outpatient clinics
- Heavy promotion by industry due to favorable profit margins
- Increasing shift of care to the home setting
  - Not just blood glucose testing, but self-monitoring of anticoagulation
  - Can be used in conjunction with telemedicine to care for patients with chronic conditions

www.cap.org
Clinics in Laboratory Testing: Point-of-Care Testing, Lewandrowski, 2009
“With the development of miniaturized devices and wireless communication, the way in which doctors care for patients will change dramatically and the role patients take in their own healthcare will increase. Health care will become more personalized through tailoring of interventions to individual patients.

The next decade will bring a new realm of precision and efficiency to the way information is transmitted and interpreted and thus the way medicine is practiced. In the future, clinicians may be able to improve the regulation of diet in infants with inborn errors of metabolism through bedside monitoring. Currently, management of such diseases requires complex testing in a hospital setting. However, researchers are developing a chemical sensor, using a small sample of blood from a finger stick, which changes color in response to metabolic irregularities. When such abnormalities are found, the diet of the infant can be adjusted immediately to prevent adverse effects such as mental retardation. “

No More Needles: A Crazy New Patch Will Constantly Monitor Your Blood

Imagine if you could always know if your glucose was low or if you were dehydrated. A new painless patch will soon send your vital signs wirelessly to your phone, giving you constant analytics on your health.

- In development by Sano Intelligence
- Sensor-laden, needle-free transdermal patch
- Goal is to monitor everything found on a basic metabolic panel
  - Prototype can measure glucose and potassium levels
- Wireless, battery-powered chip (7 day lifespan)
- Collect and transmit data to mobile phone or other computing device
- Materials cost $1-2/per sensor
- Gearing up for a pilot study and could be ready to launch next year

http://www.fastcoexist.com/1680025/no-more-needles-a-crazy-new-patch-will-constantly-monitor-your-blood
http://www.darkdaily.com/transdermal-patch-continuously-monitors-blood-chemistry%e2%80%94without-needles-and-clinical-pathology-laboratory-testing-1031#axzz2Au9KZMXK
WILL POCT REPLACE THE CLINICAL LABORATORY?

- Not likely
  + While more tests currently in the laboratory will move to the POC, new highly complex and esoteric tests will replace them
  + However, the movement to increase POCT could help ameliorate the shortage of qualified medical technologists

- Factors that might decrease POCT
  + Increased regulation
  + Cost considerations and reimbursement issues
Back to the article...

“As technology advances and gets more portable, I see us bringing more tools to the bedside, and therefore spending more time with patients, instead of sending them hither and thither to diagnostic suites. The more time with the patient, the better. This is how you will know us, the doctors of the next millennium: by the things we carry.”

Applies not just to physicians, but also to point of care testing.
THANK YOU VERY MUCH FOR YOUR ATTENTION.

amferguson@cmh.edu