Saliva Diagnostics for Kidney Disease

Linden Heflin & Sarah Walsh

May 3, 2007
Overview

- Advantages of Saliva Diagnostics
- Background
- FDA Approval
- Consumer Satisfaction
- Consumer Preference
- Pricing and Demand
- Net Present Worth
Alternative to Blood Testing

- Saliva contains many of the components of blood
  - In much lower concentration
  - Mostly items that passively diffuse through salivary glands

- Collecting saliva is much less invasive and faster
  - Reduces risk of exposure for health care professional
  - Saliva has fewer components that interfere with assay, reducing steps needed in analysis

- Some people refuse blood testing for cultural or religious reasons
The Beginning of Saliva Diagnostics

- **Microfluidics**
  - A new field which began in the early 90’s
  - Combination of physics, chemistry, biotechnology, and engineering
  - Develops a better understanding of how fluids move on a micro and nanoliter scale
    - Allows for the design of more sensitive diagnostic devices
Current State of Saliva Diagnostics

- Conditions presently being assessed using saliva
  - Alcohol consumption
  - Drug use
  - Hormone levels
  - HIV 1 and 2
  - Viral hepatitis A, B, and C

- Current research
  - Cardiovascular disease
  - Cancer
  - Alzheimer’s
  - Osteoporosis
Saliva

• Saliva has many components
  - Mostly water with some mucus
  - A variety of electrolytes (K⁺, Na⁺, Cl⁻, Ca⁺)
• Many proteins found in blood also make their way into saliva
Saliva Composition

- 98% water
- Electrolytes
  - Sodium ~32 mmol/L
  - Potassium ~22 mmol/L
  - Calcium ~1.7 mmol/L
  - Magnesium ~0.18 mmol/L
  - Copper ~0.4 µmol/L
  - Lead ~0.55 µmol/L
  - Cobalt ~1.2 µmol/L
  - Strontium ~1 µmol/L
  - Hydrogen Carbonate ~20 mmol/L
  - Iodide ~10 µmol/L
  - Bromide ~14 mmol/L
  - Hypothiocyanate ~1.2 µmol/L
  - Nitrate ~1.1 µmol/L
  - Nitrite ~178 µmol/L
  - Fluoride ~68 µmol/L
  - Sulfate ~5.8 µmol/L
- Mucus
  - Mucopolysaccharides
  - Glucose ~175 µmol/L
- Metabolites
  - Bilirubin ~15 µmol/L
  - α-ketoglutaric acid ~2.4 µmol/L
  - Pyruvic acid ~75 µmol/L
- Proteins
  - α-amylase ~650–800 µg/ml
  - Peroxidase ~5–6 µg/ml
  - Secretory IgA ~96–102 µg/ml
  - Lactoferrin ~1–2 µg/ml
  - Fibronectin ~0.2–2 µg/ml
- Cells

5/2/2007
Our Screening Procedure

- Can biomarker be detected in saliva
- Abnormal levels indicate threat of organ malfunction
- How do you detect abnormal levels
- How accurate are detection methods
- How widely applicable are detection methods
- How helpful is result in medical decision making
- Is test effective in early diagnosis (compared to serum testing)
- Weigh accuracy vs. speed, convenience, portability
- Cost of detection method
- Making product attractive to consumer
Screening Procedure Flow Chart

1. **No, back to beginning**
2. **Does organ malfunction have salivary biomarkers?**
   - Yes, investigate possibility of false negatives
   - **No, back to beginning**
3. **Is it accurate enough?**
4. **Can it compete with serum tests?**
   - Serum tests more desirable
   - **No, back to beginning**
   - Essentially equal
   - **No, back to beginning**
   - **Saliva is superior**
     - **Accuracy**
     - **Patient Comfort**
     - **Speed**
     - **Is it useful for early diagnosis?**
6. **Useful as precursor to more invasive tests**
7. **Preliminary Economic Analysis**
   - **Profitable**
   - **Not Profitable, back to beginning**
   - **FDA Pre-Market Notification**
8. **5/2/2007**
9. **9**
The kidney’s responsibility is to clean the blood.

Most waste in blood passively diffuses in the kidney, just like most of saliva’s components come from passive diffusion.

About 1 in 12 people have some kidney disease:
- 9th leading cause of death in USA
- 80,000 deaths per year

About 450,000 people depend on dialysis or kidney transplants to live.
Symptoms of Kidney Disease

- High blood pressure
- Fatigue, less energy
- Poor concentration and appetite
- Trouble sleeping and night time muscle cramps
- Swollen feet and ankles
- Puffiness around eyes, particularly in the morning
- Dry, itchy skin
- Frequent urination
Creatinine Test

- Typically ordered as part of a general metabolic panel
- Usually tested for in urine and serum, but correlations now exist between serum creatinine and salivary levels of creatinine
- Has certain ranges that are considered healthy
  - Correlations exist that relate serum creatinine to saliva creatinine and also to glomerular filtration rate (GFR)
  - The GFR is a good indicator of kidney disease progression
- The physician is looking for the creatinine clearance
Creatinine and GFR

- Creatinine is a break down product of creatinine in muscle
- The kidney removes it from the blood
- Presence may indicate kidney failure or dysfunction
- Correlations exist relating it to Glomerular Filtration Rate
  - GFR mL/min/1.73m²
  - Creatinine mg/dL

Cockcroft-Gault Equation:

\[
GFR = \frac{(140 - Age) \cdot Mass}{815 \cdot P_{cr}}
\]

\[
P_{cr} = 10S_{cr}
\]

\(P_{cr}\) = Plasma Creatinine Concentration (mmol/L)
\(S_{cr}\) = Saliva Creatinine Concentration (mmol/L)
Mass in kilograms
### Unhealthy GFR

<table>
<thead>
<tr>
<th>GFR</th>
<th>Stage</th>
<th>Description</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>90+</td>
<td>1</td>
<td>Normal kidney function</td>
<td>Observe, control blood pressure</td>
</tr>
<tr>
<td>60–89</td>
<td>2</td>
<td>Mildly reduced kidney function, with urine abnormalities, indicates kidney disease</td>
<td>Find out why kidney function is reduced</td>
</tr>
<tr>
<td>30–59</td>
<td>3</td>
<td>Moderately reduced kidney function</td>
<td>Make a diagnosis with additional testing</td>
</tr>
<tr>
<td>15–29</td>
<td>4</td>
<td>Severely reduced kidney function</td>
<td>Plan for endstage renal failure</td>
</tr>
<tr>
<td>14 down</td>
<td>5</td>
<td>Endstage kidney failure</td>
<td>Dialysis and/or transplant</td>
</tr>
</tbody>
</table>

\[
P_{cr} = 10 \, S_{cr} \quad \quad \quad GFR = \frac{(140 - \text{Age}) \cdot \text{Mass}}{8150 \cdot S_{cr}}
\]

5/2/2007
Description of Assay

- The test utilizes the Jaffé reaction, which requires certain reagents
  - NaOH to provide alkalinity
  - Picric Acid to react with the creatinine

- The Picric Acid produces a color change upon reaction
  - The color change can be tracked with spectrophotometry

- Serum tests require more reagents to reduce interference
Saliva vs. Blood Collection

• Blood collection
  - Requires invasive, expensive needle
  - Requires disinfection

• Saliva collection
  - Only requires spitting into a vial
  - Patient needs to rest, not eating, for 5 minutes prior to collection
  - Patient must chew inert paraffin gum for 1 minute to stimulate saliva flow
FDA Approval

- FDA approval is an important part of medical device development
- According to FDA regulations, a salivary creatinine test is considered a medical device
- Medical devices are regulated by the FDA’s Center for Devices and Radiological Health
- First step in the approval process is to classify the device
- There are three classifications, requiring different degrees of approval processes
- Creatinine tests fall into Category II
  - Does not require Pre-Market Approval
  - Requires Pre-Market Notification
Pre-Market Notification

- All medical devices being brought to market must submit a Pre-Market Notification

- The Pre-Market Notification must establish Substantial Equivalence:
  - has the same intended use as the predicate; and
  - has different technological characteristics and the information submitted to FDA;
    - does not raise new questions of safety and effectiveness; and
    - demonstrates that the device is at least as safe and effective as the legally marketed device.
Other Requirements

• Good Manufacturing Practices/Quality System Regulation

• Provides guidance for:
  – Designing processes and products
  – Process control
  – Employee training
  – Facilities
  – Labeling
  – Distributing
Consumer Satisfaction

- Relate “consumer” properties to physical properties
  - Sensitivity
    - Ability to detect creatinine
  - Likelihood for False Positives
    - Due to positive interference
  - Likelihood for False Negatives
    - Due to bilirubin interference
  - Discomfort
    - Associated with obtaining sample blood vs. saliva

- Consumers are both patients and medical professionals
\[ H_i = \sum_j w_{i,j} y_{i,j} \]

- \( H_2 \): consumer satisfaction with existing product
- \( H_1 \): consumer satisfaction with new product
- \( w \): weight of property \( j \) for product \( i \)
- \( y \): satisfaction with property \( j \) for product \( i \)

- \( H \) is a function of consumer properties related to physical properties
Weights for Satisfaction Function

- Weights were determined from consumer surveys
- Participants were asked to rate the following factors
  - Discomfort
  - Sensitivity
  - Chance for False Positive Results
  - Chance for False Negative Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discomfort</td>
<td>0.22</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.25</td>
</tr>
<tr>
<td>False Negative Rate</td>
<td>0.26</td>
</tr>
<tr>
<td>False Positive Rate</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Discomfort

- Discomfort is a consumer property related to the invasiveness of the test.

- Discomfort ($D$) is a constant dependent on whether or not blood is drawn and is added to the satisfaction function.
  
  $D = 0.5$ if blood is drawn
  
  $D = 1$ if no blood is drawn
Consumer satisfaction corresponds to the disease stage that the test can detect.
The ability of the test to detect certain disease stages relates to the minimum detectable concentration of the test.
Sensitivity

Consumer Satisfaction vs. Concentration

Consumer satisfaction decreases with increasing minimum detectable concentration
Interference

- Certain compounds are known to interfere with the Jaffé reaction and create misleading results

### Positive Interference
- Cause creatinine test results to be higher than actual
  - *Interfering compounds*
    - Pyruvic acid, glucose, and alpha-ketoglutaric acid
      - Less significant

### Negative Interference
- Cause creatinine test results to be lower than actual
  - *Interfering compound*
    - Bilirubin
      - Moderately significant

5/2/2007
Distribution of Creatinine in Patients

Percent of Patients with Specific Salivary Creatinine Concentrations

Creatinine Concentration (umol/L)

% Patients

12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99 102 105

5/2/2007
Satisfaction dramatically drops as the percent of false positives increases.
The percentage of tests that give a false positive increases with an increasing concentration of interfering compounds.
Satisfaction decreases with the concentration of interfering compounds.
Positive Interference

• Challenge
  – Compounds such as glucose can cause the test to show slightly higher creatinine levels than are actually present
  – Occurrence of false positives is ~1%

• Possible Resolution
  – Include monitors to measure levels of positively interfering compounds
    • i.e. a glucose meter
Negative Interference

Consumer Satisfaction vs. Percent False Negative

Satisfaction decreases rapidly with the percent false negative
A higher bilirubin to creatinine concentration ratio indicates higher interference
Negative Interference

• Challenge
  – Excessive amounts of bilirubin cause tests to show lower creatinine levels than are actually present

• Resolution
  – Sodium dodecyl sulfate decreases the effects of bilirubin, thereby reducing the likelihood for false negative results
Negative Interference

The percentage of false negative results decreases with increasing SDS concentration up to 140 mmol/L.
Negative Interference

Consumer Satisfaction vs. [SDS]

Satisfaction increases with increasing SDS concentration
Pricing and Demand Model

\[ \Phi(d_1) = p_1 d_1 - \left( \frac{\alpha}{\beta} \right)^\rho \frac{Y - p_1 d_1}{p_2} \left[ \frac{Y - p_1 d_1}{p_2} \right]^{1-\rho} d_1^\rho = 0 \]

- \( p_1 \): new product price
- \( d_1 \): new product demand
- \( \alpha \): describes consumer knowledge of new product
- \( \beta \): describes consumer preference for new product
- \( Y \): total consumer budget
- \( p_2 \): existing product price (serum test – $10/test)
- \( \rho \): constant of 0.75
Awareness Function

\[ \Phi (d_1) = p_1 d_1 \left[ \begin{array}{c} \alpha \\ \beta \end{array} \right] ^{\rho} p_1 \left[ \frac{Y - p_1 d_1}{p_2} \right]^{1-\rho} d_1^\rho = 0 \]

- Awareness (\( \alpha \)) is a function of consumer awareness of the product
- Awareness increases with time to a value of 1, indicating total awareness
Advertising and education can increase awareness

\[ \Phi(d_1) = p_1 d_1 - \begin{pmatrix} \alpha \\ \beta \end{pmatrix} p_1 \left[ \frac{Y - p_1 d_1}{p_2} \right]^{1-\rho} d_1^\rho = 0 \]
Influence of Awareness

Demand vs. Price for the First Three Years of Project
Without SDS or Glucose Monitor

As the consumer awareness increases for the first three years, the demand for the product increases.
Beta Function

\[ \Phi(d_1) = p_1 d_1 - (\frac{\alpha}{\beta})^\rho p_1 \left[ \frac{Y - p_1 d_1}{p_2} \right]^{1-\rho} d_1^\rho = 0 \]

\[ \beta = \frac{H_2}{H_1} \]

- \( H_1 \): Consumer satisfaction for the new product
- \( H_2 \): Consumer satisfaction for the existing product

-Lower beta values indicate a more appealing product

*Beta does not factor in price*
Product Design

- **Minimum Detectable Concentration**
  
  - Low
    - 16.8 umol/L
    - Stages 2, 3, 4, and 5
    - Spectrophotometer
    - Includes Standards

  - High
    - 40 umol/L
    - Stages 4 and 5
    - Determined Visually
    - Standards Unnecessary

- **Inclusion of Anti–Interference Components**
  
  - Option 1: No Additives
  
  - Option 2: SDS included to counter negative interference

  - Option 3: Glucose meter to monitor positive interference

  - Option 4: SDS and glucose meter counter interference
Consumer Satisfaction vs. Detectable Concentration for Various Interference Scenarios

Satisfaction is higher for options including additives to counteract interference
Consumer Preference

Beta Values Used for Economic Analysis

- With SDS and Glucose Meter (D.C.=16.8 umol/L)
- With SDS (D.C.=16.8 umol/L)
- With Glucose Meter (D.C.=16.8 umol/L)
- No Additives (D.C.= 16.8 umol/L)
- No Additives (D.C.=40 umol/L)

\[ \Phi(d_1) = p_1d_1 - \left( \frac{\alpha}{\beta} \right)^\rho p_1 \left[ \frac{Y - p_1d_1}{p_2} \right]^{1-\rho} d_1^\rho = 0 \]
The demand is higher for the test with a lower minimum detectable concentration.
The test able to detect low concentrations was more profitable despite added product cost.
Demand versus price curves were created using the pricing and demand model for the four interference scenarios.

5/2/2007
Net Present Worth Over Three Year Period for Various Product Options at Low Minimum Detectable Concentration

- **Nothing Added**: Beta = 0.63
- **With SDS**: Beta = 0.46
- **With Glucose Monitor**: Beta = 0.50
- **With SDS and Glucose Monitor**: Beta = 0.39

The most profitable design is the product with SDS priced at $4/test.
Conclusions

• The product design including SDS yields the highest NPW

• The product with the lowest beta value was not the most profitable
Acknowledgements

• Dr. Miguel Bagajewicz
  – CBME, University of Oklahoma
• Dr. Stephen Kastl
• Noah Abbas
References

10. Levey, A; Bosch, J; Lewis, J; Greene, T; Rogers, N; Roth, D: "A More Accurate Method to Estimate Glomerular Filtration Rate from Serum Creatinine: A New Prediction Equation." Annals of Internal Medicine 1999; 130(6): 461–470
12. http://adr.iadrjournals.org/cgi/content/full/18/1/3