Saliva Diagnostics for Kidney Disease

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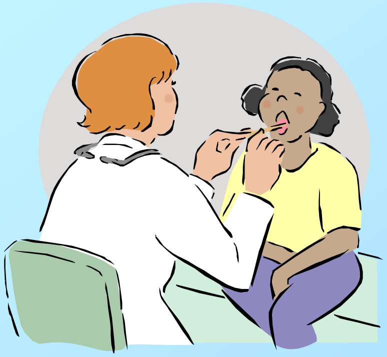
Overview

- 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

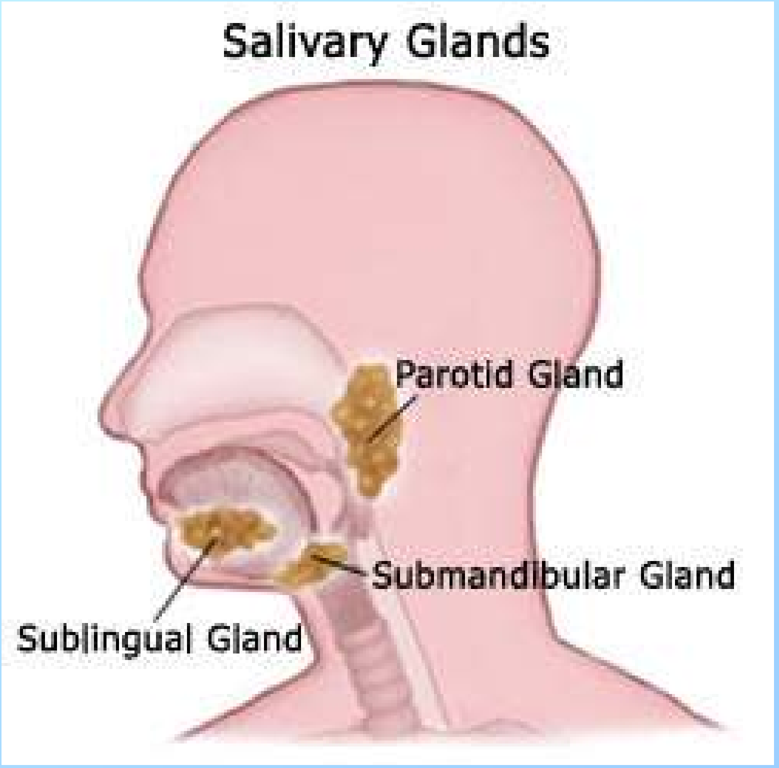
- Microfluidics
  - A new field which began in the early 90's
  - Combination of physics, chemistry, biotechnology, and engineering
  - Develops a better understanding of how fluids move on a micro and nanoliter scale
    - Allows for the design of more sensitive diagnostic devices

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



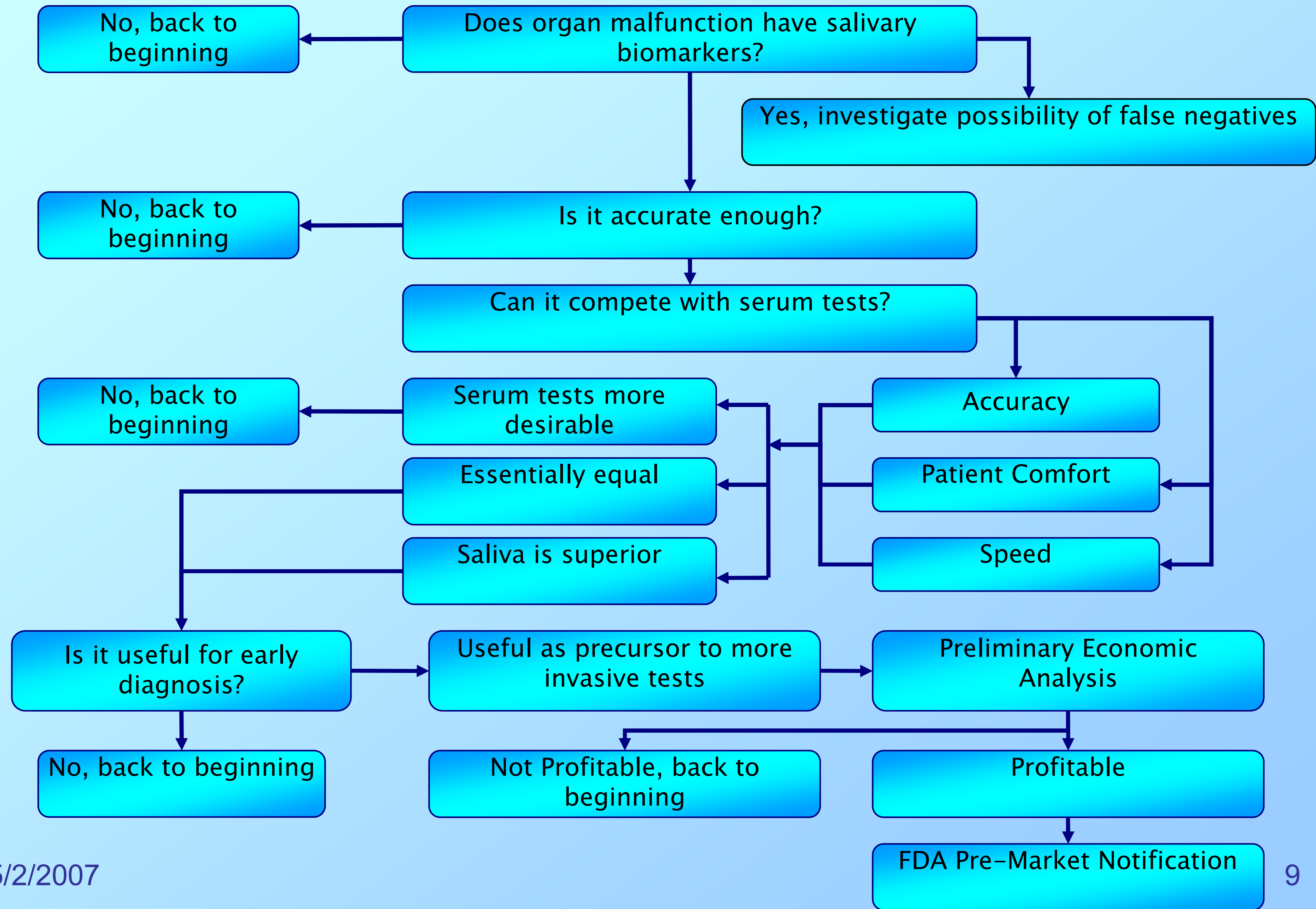
Saliva Composition

- 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

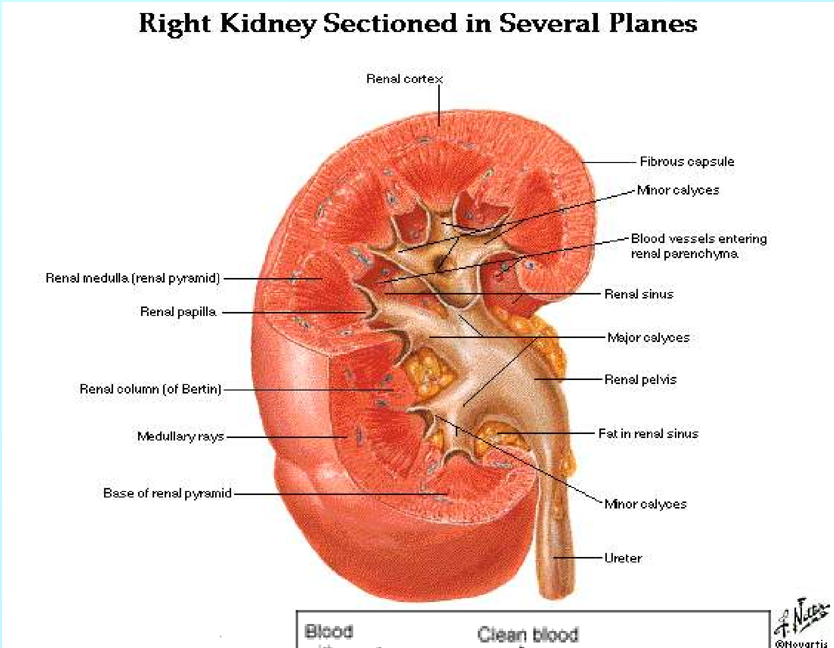
Our Screening Procedure

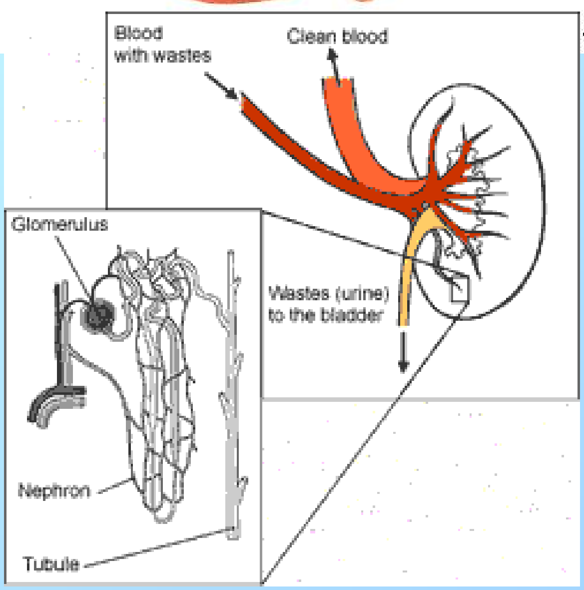
- Can biomarker be detected in saliva
- Abnormal levels indicate threat of organ malfunction
- How do you detect abnormal levels
- How accurate are detection methods
- How widely applicable are detection methods
- How helpful is result in medical decision making
- Is test effective in early diagnosis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

Screening Procedure Flow Chart



The Kidney

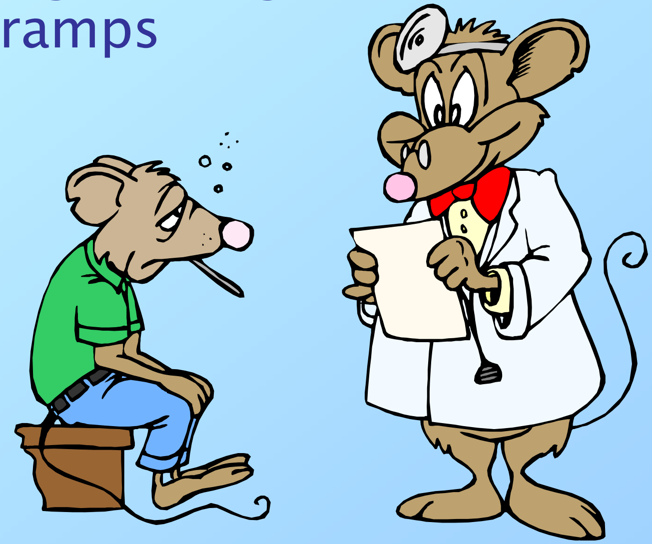




- The kidney's responsibility is to clean the blood
- Most waste in blood passively diffuses in the kidney, just like most of saliva's components come from passive diffusion
- About 1 in 12 people have some kidney disease
  - 9th leading cause of death in USA
  - 80,000 deaths per year
- About 450,000 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

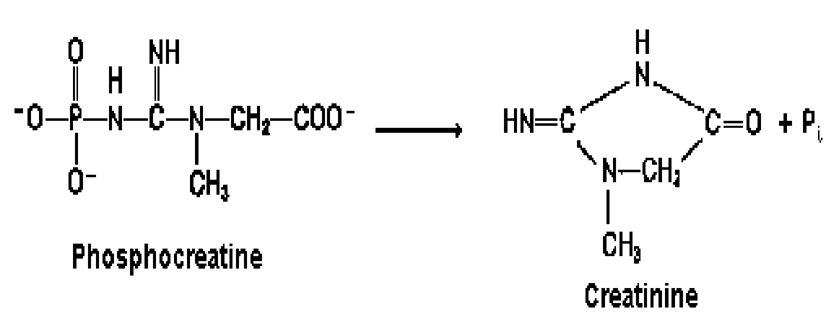


Creatinine Test

- 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 the 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/1.73m^2
  - Creatinine mg/dL



Cockcroft-Gault Equation:

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

$$P_{cr} = 10 S_{cr}$$

P_{cr}=Plasma Creatinine Concentration (mmol/L)

S_{cr}=Saliva Creatinine Concentration (mmol/L)

Mass in kilograms

Unhealthy GFR

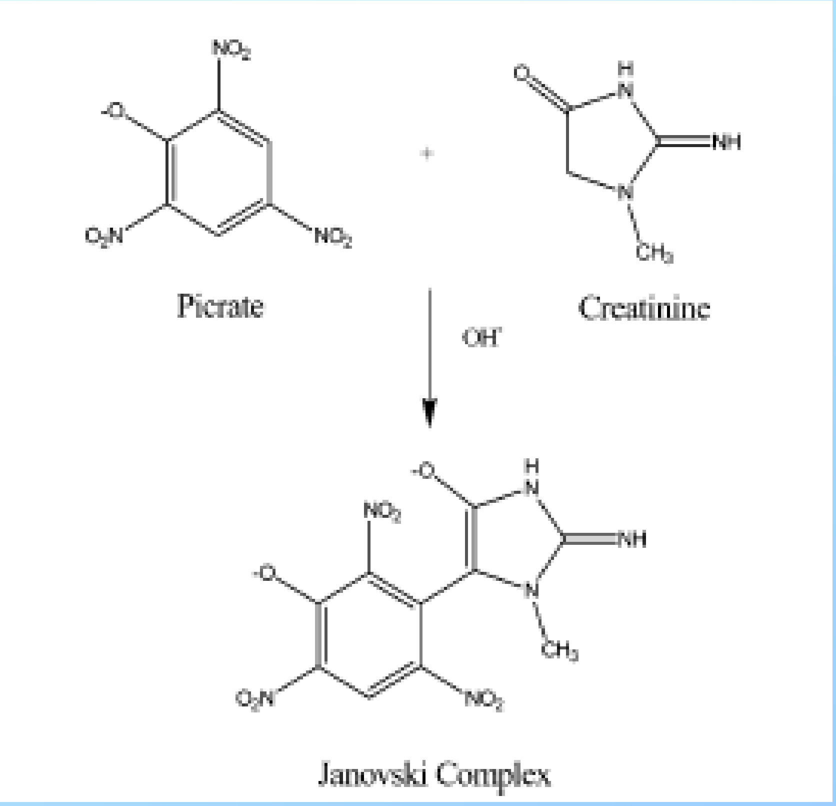
GFR	Stage	Description	Treatment
90+	1	Normal kidney function	Observe, control blood pressure
60-89	2	Mildly reduced kidney function, with urine abnormalities, indicates kidney disease	Find out why kidney function is reduced
30-59	3	Moderately reduced kidney function	Make a diagnosis with additional testing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

$$P_{cr} = 10\, S_{cr}$$

$$GFR = \frac{(140 - Age) \cdot Mass}{8150 \cdot S_{cr}}$$

Description of Assay

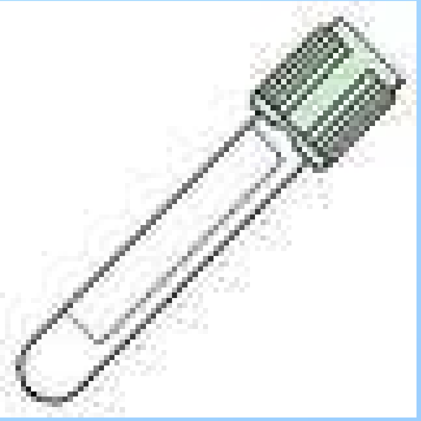
- The test utilizes the Jaffé reaction, which requires certain reagents
  - NaOH to provide alkalinity
  - Picric Acid to react with the creatinine
- The Picric Acid produces a color change upon reaction
  - The color change can be tracked with spectrophotometry
- Serum tests require more reagents to 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

U.S. Food and Drug Administration

U.S. Department of Health and Human Services

- 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

Consumer Satisfaction Model

$$H_i = \sum_j w_{i,j} y_{i,j}$$

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

Parameter	Weight
Discomfort	0.22
Sensitivity	0.25
False Negative Rate	0.26
False Positive Rate	0.27

Discomfort

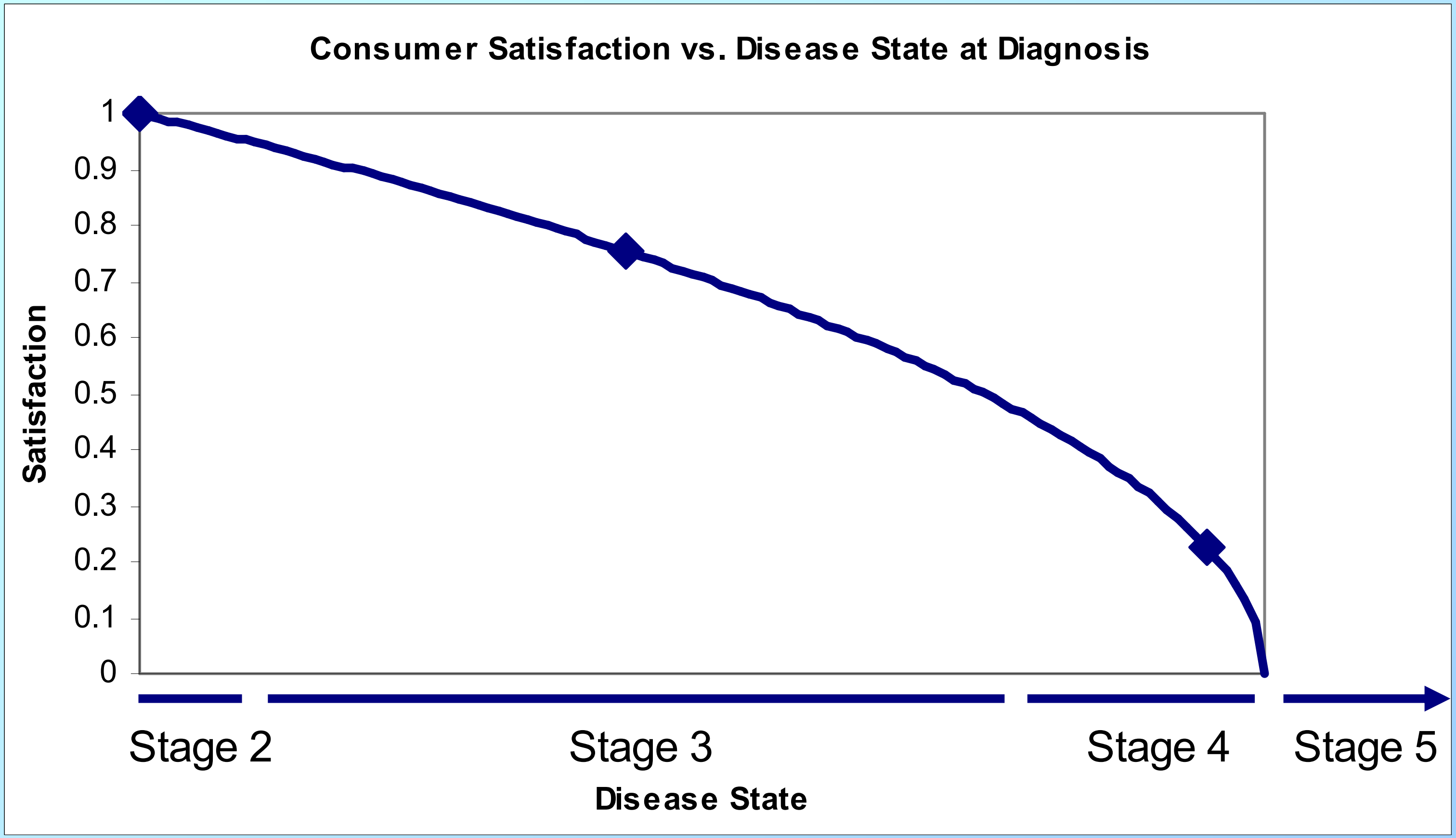
- Discomfort is a consumer property related to the invasiveness of the test

- 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

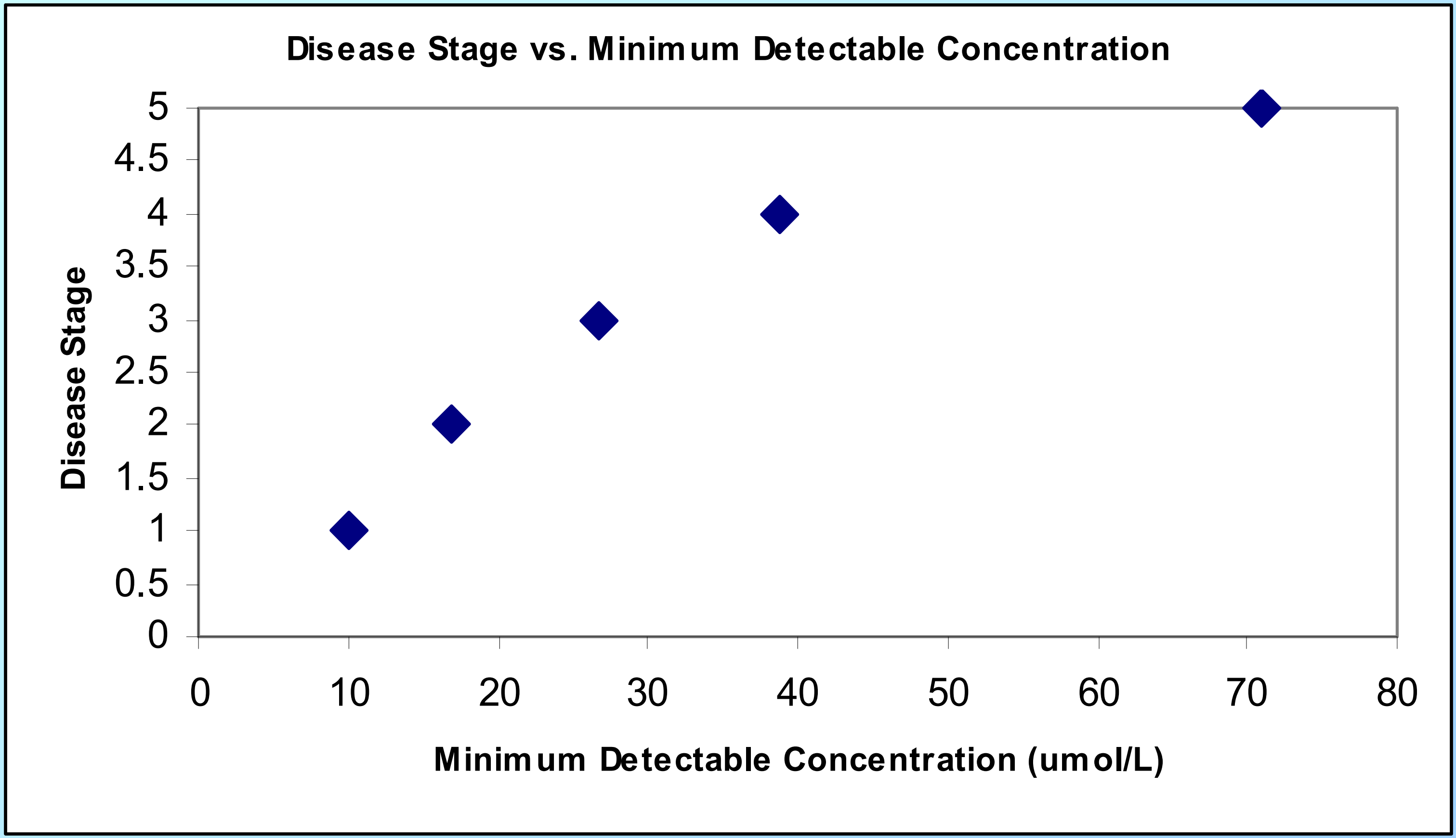
D = 1 if no blood is drawn

Sensitivity



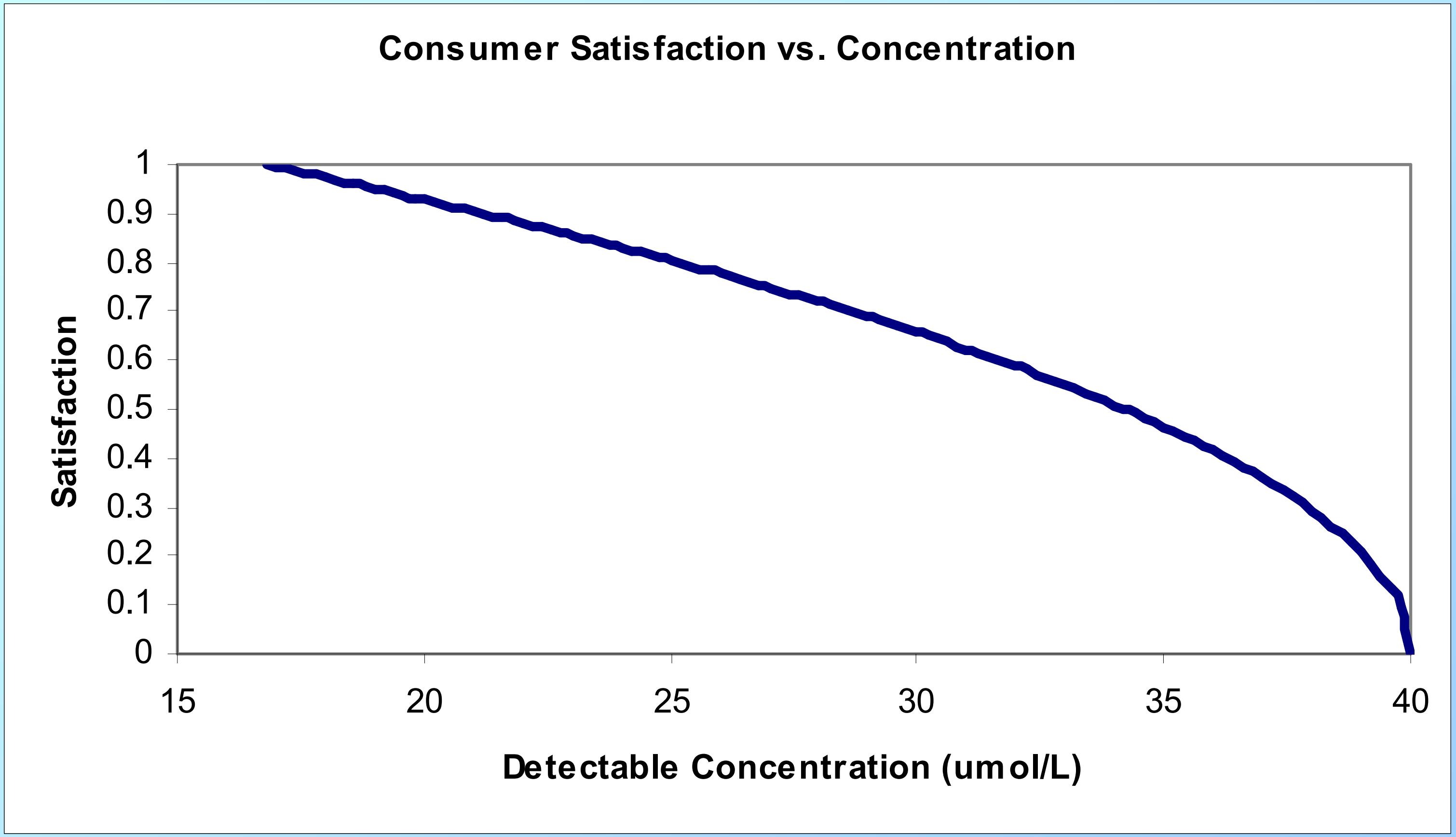
Consumer satisfaction corresponds to the disease stage that the test can detect

Sensitivity



The ability of the test to detect certain disease stages relates to the minimum detectable concentration of the test

Sensitivity



Consumer satisfaction decreases with increasing minimum detectable concentration

Interference

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

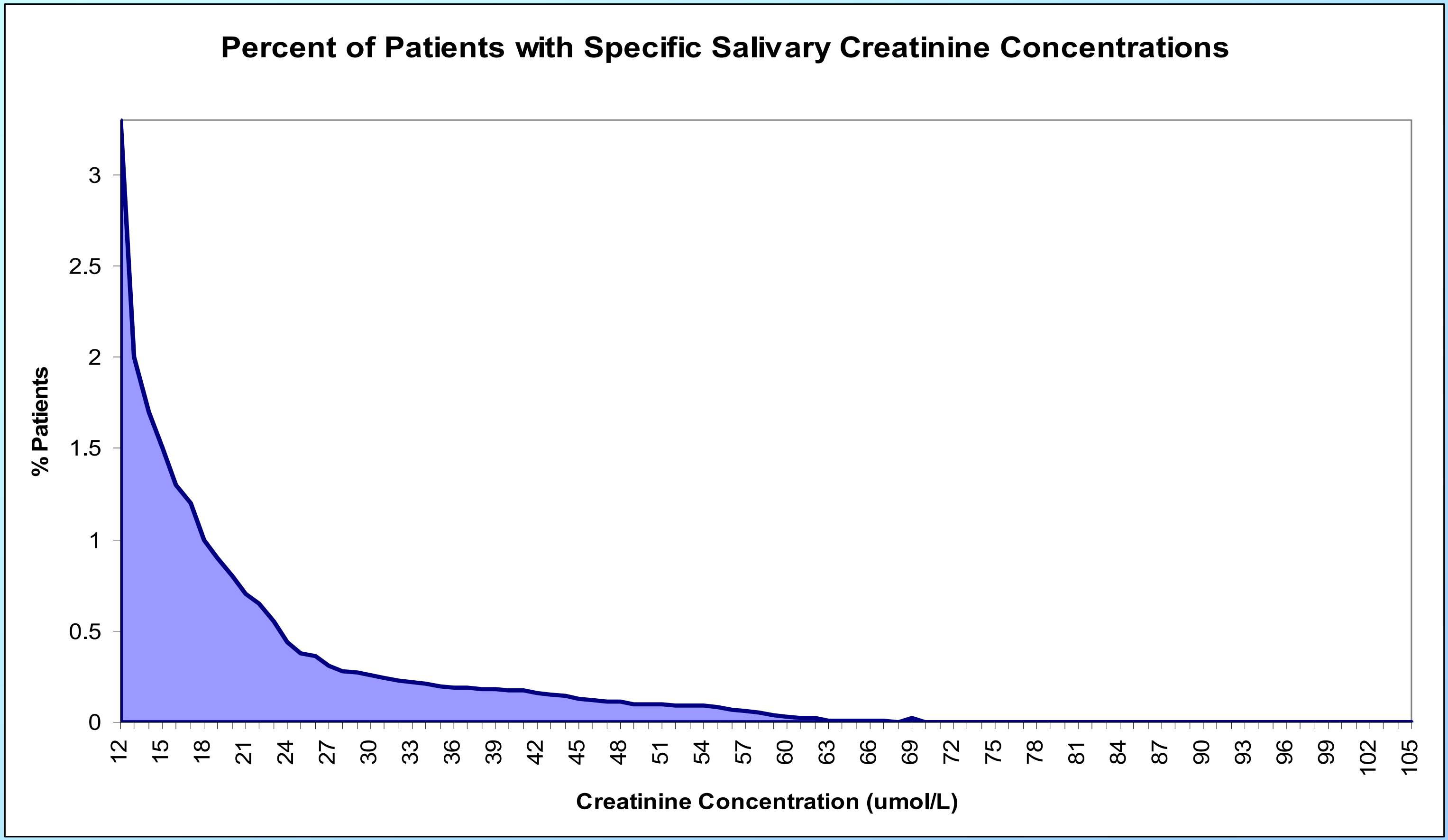
Positive Interference

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

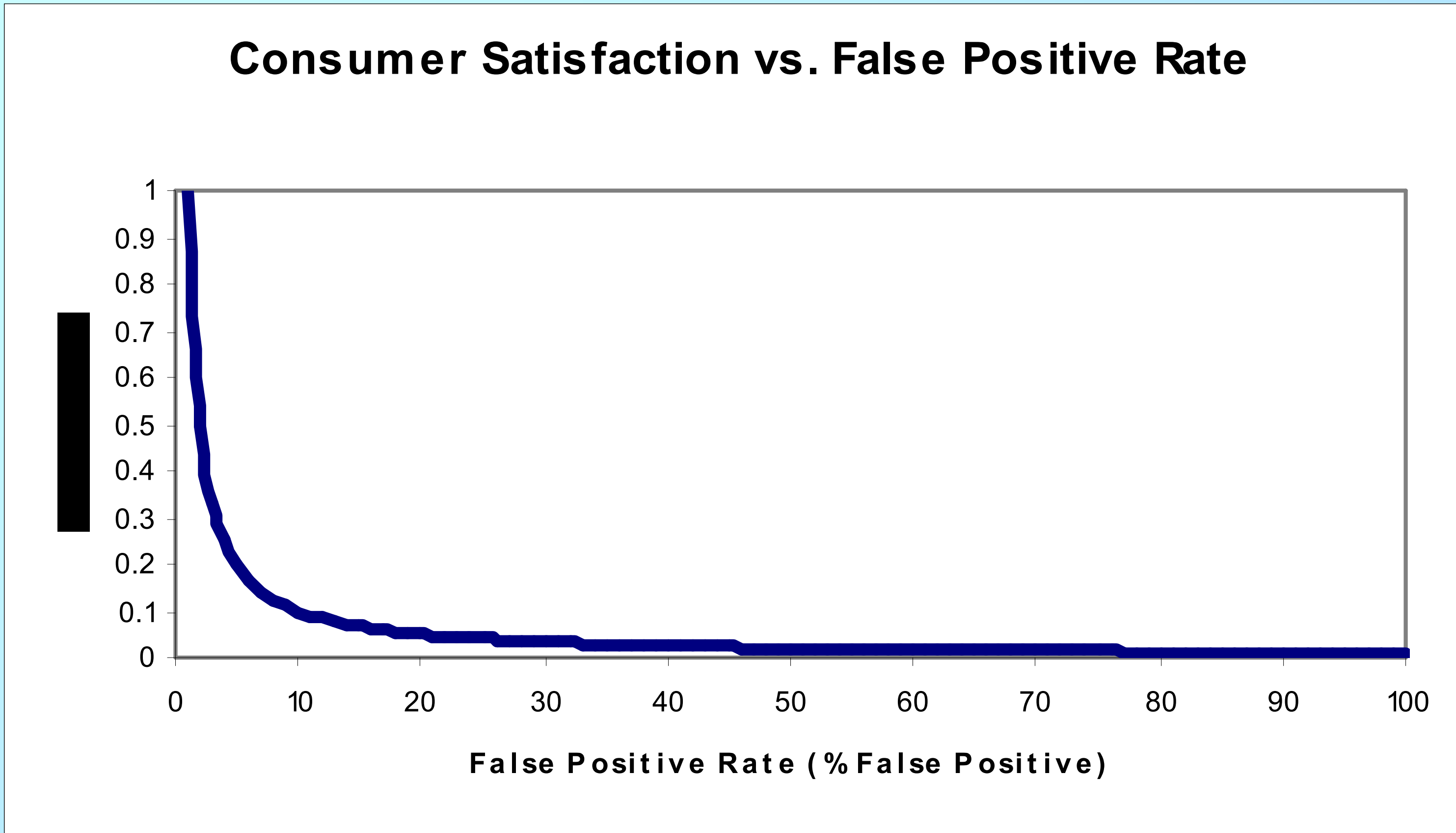
Negative Interference

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

Distribution of Creatinine in Patients

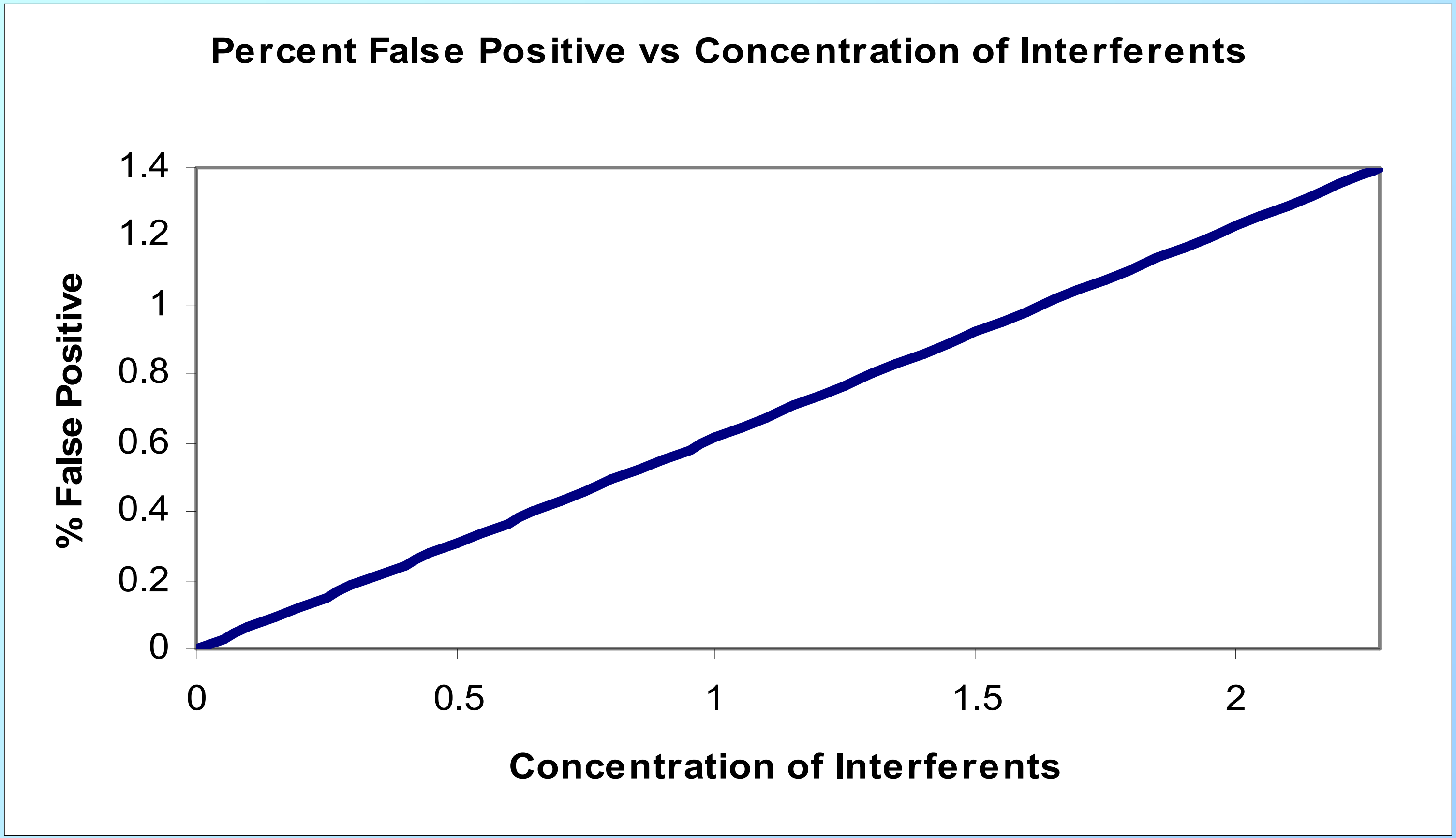


Positive Interference



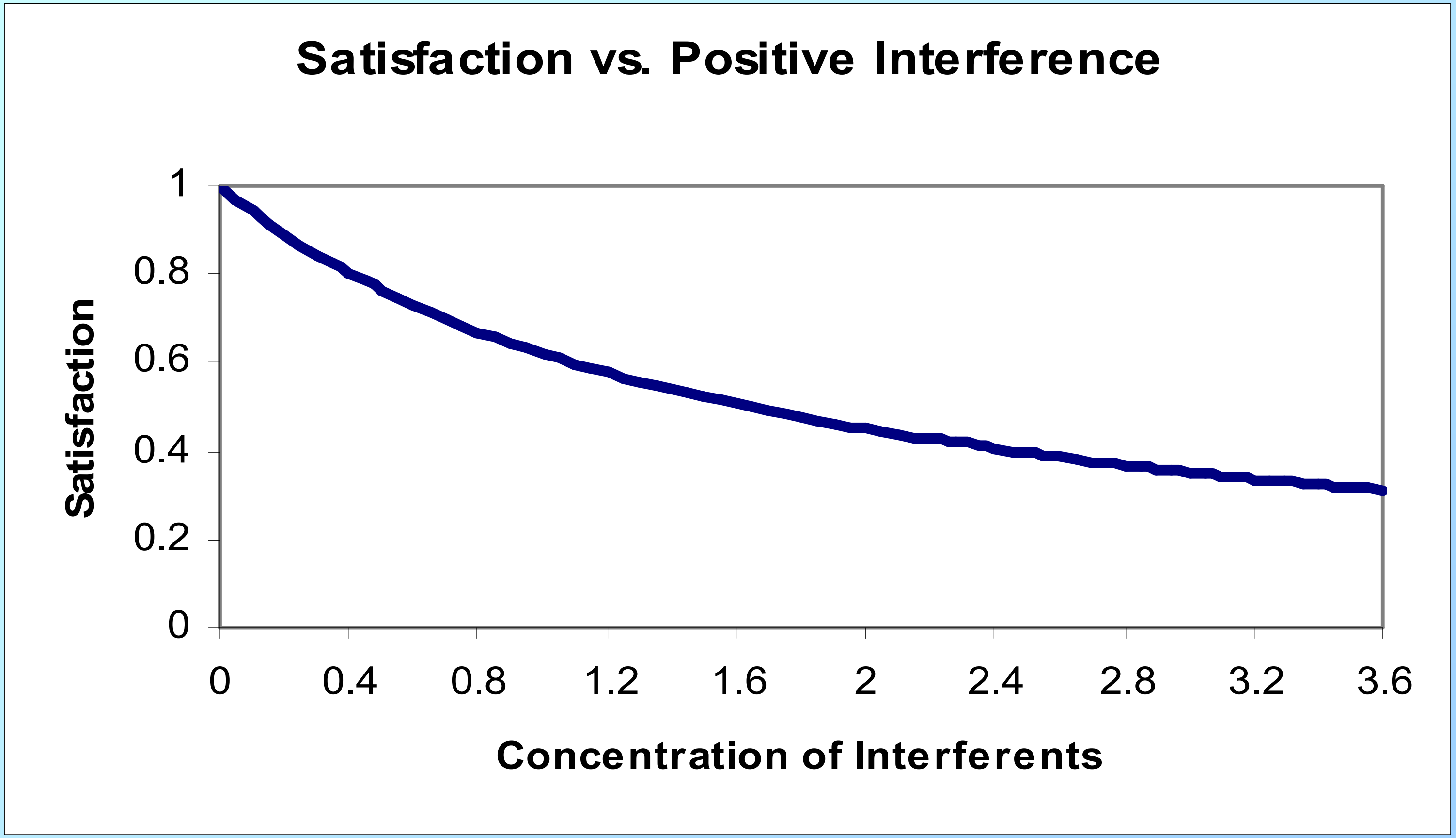
Satisfaction dramatically drops as the percent of false positives increases

Positive Interference



The percentage of tests that give a false positive increases with an increasing concentration of interfering compounds

Positive Interference

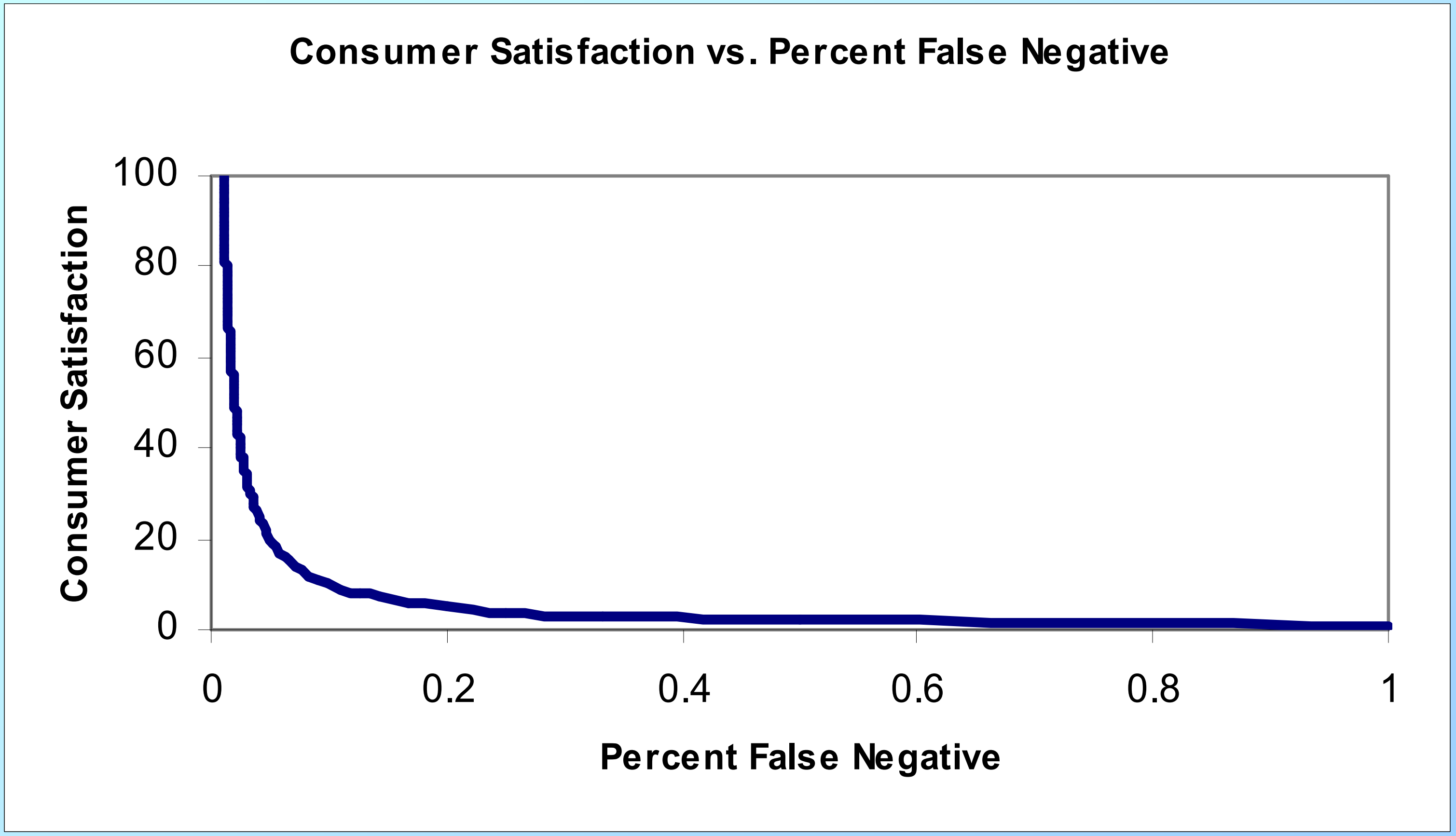


Satisfaction decreases with the concentration of interfering compounds

Positive Interference

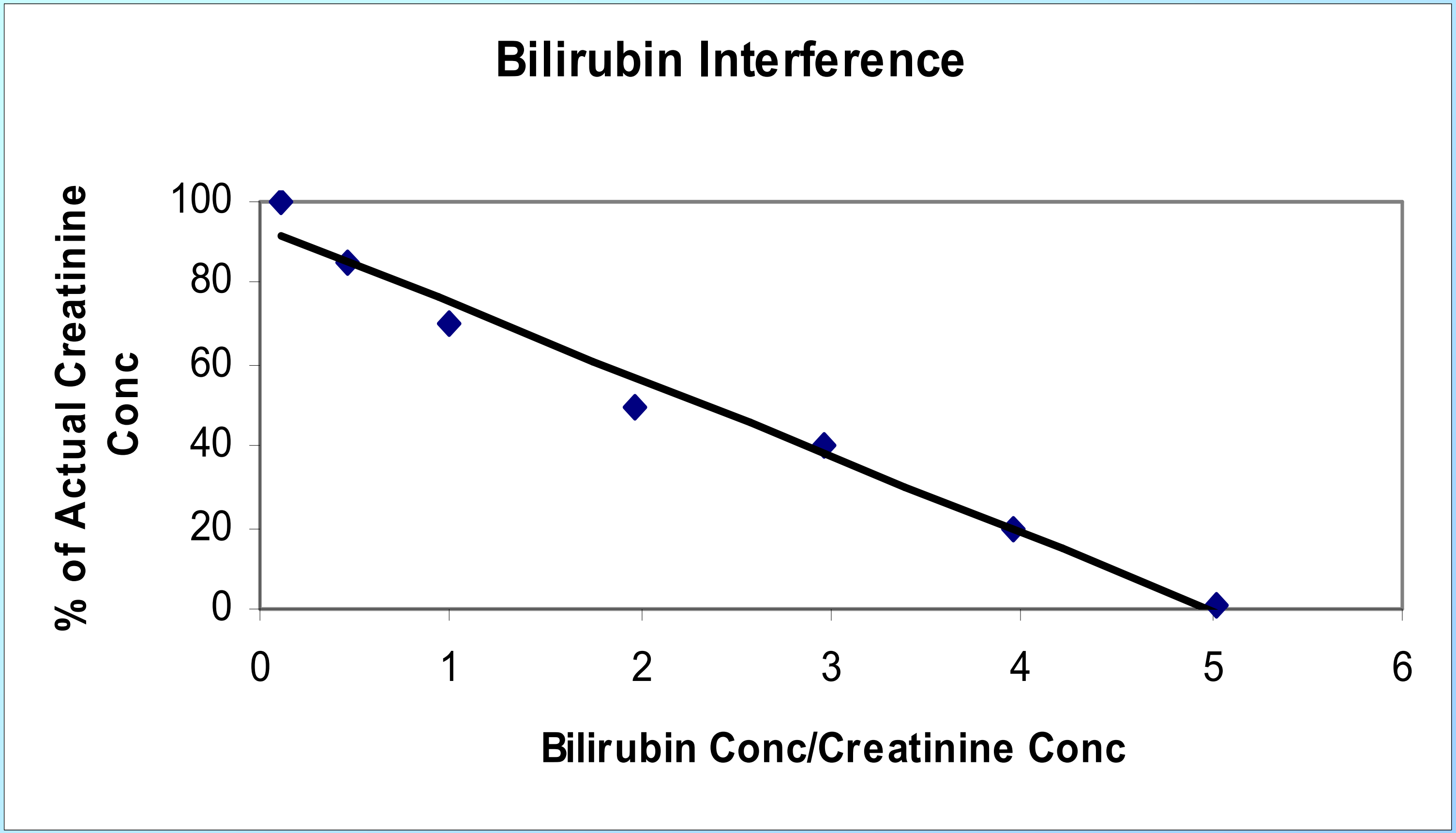
- Challenge
  - Compounds such as glucose can cause the test to show slightly higher creatinine levels than are actually present
  - Occurrence of false positives is ~1%
- Possible Resolution
  - Include monitors to measure levels of positively interfering compounds
    - i.e. a glucose meter

Negative Interference



Satisfaction decreases rapidly with the percent false negative

Negative Interference



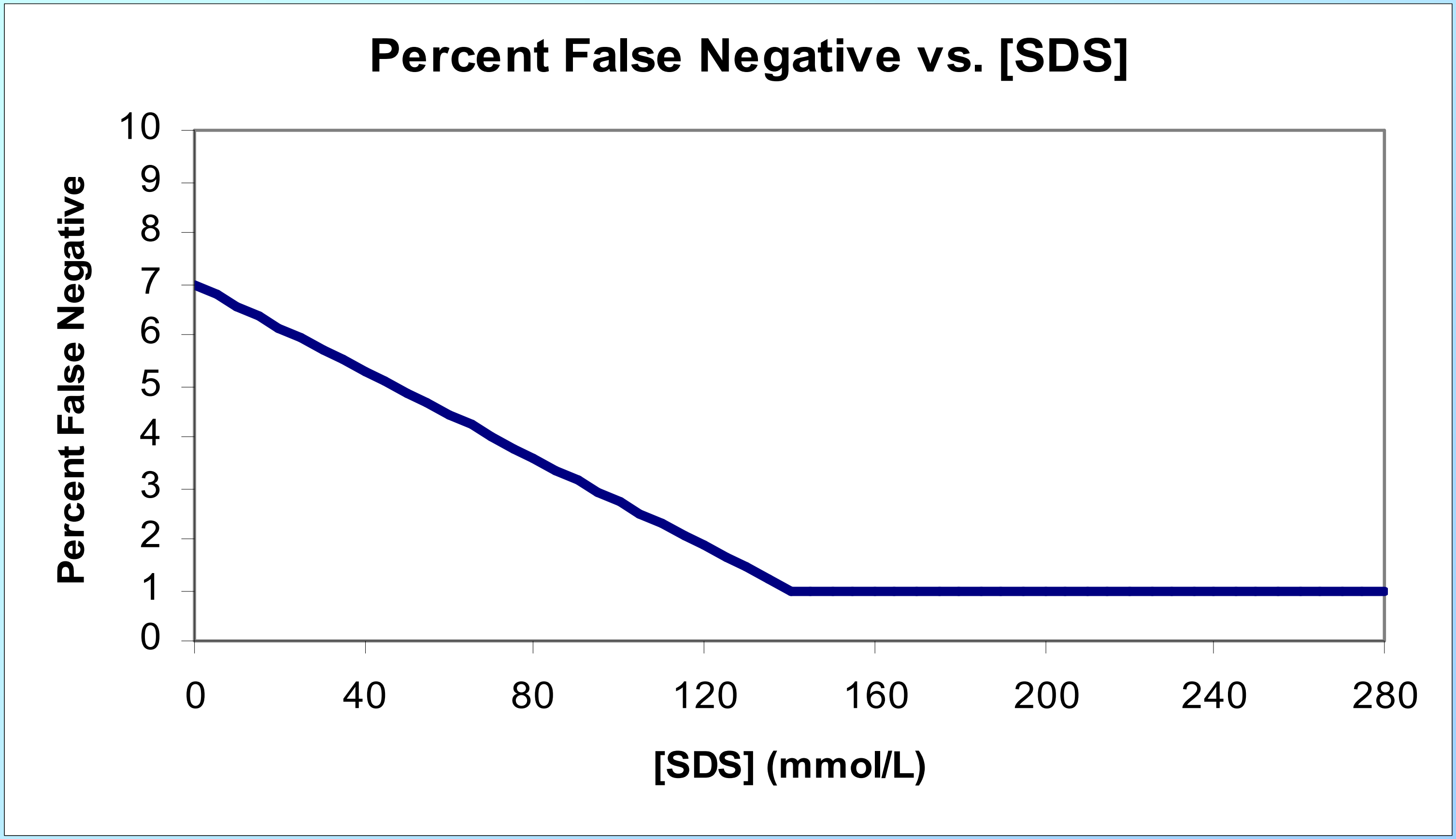
A higher bilirubin to creatinine concentration ratio indicates higher interference

Negative Interference

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

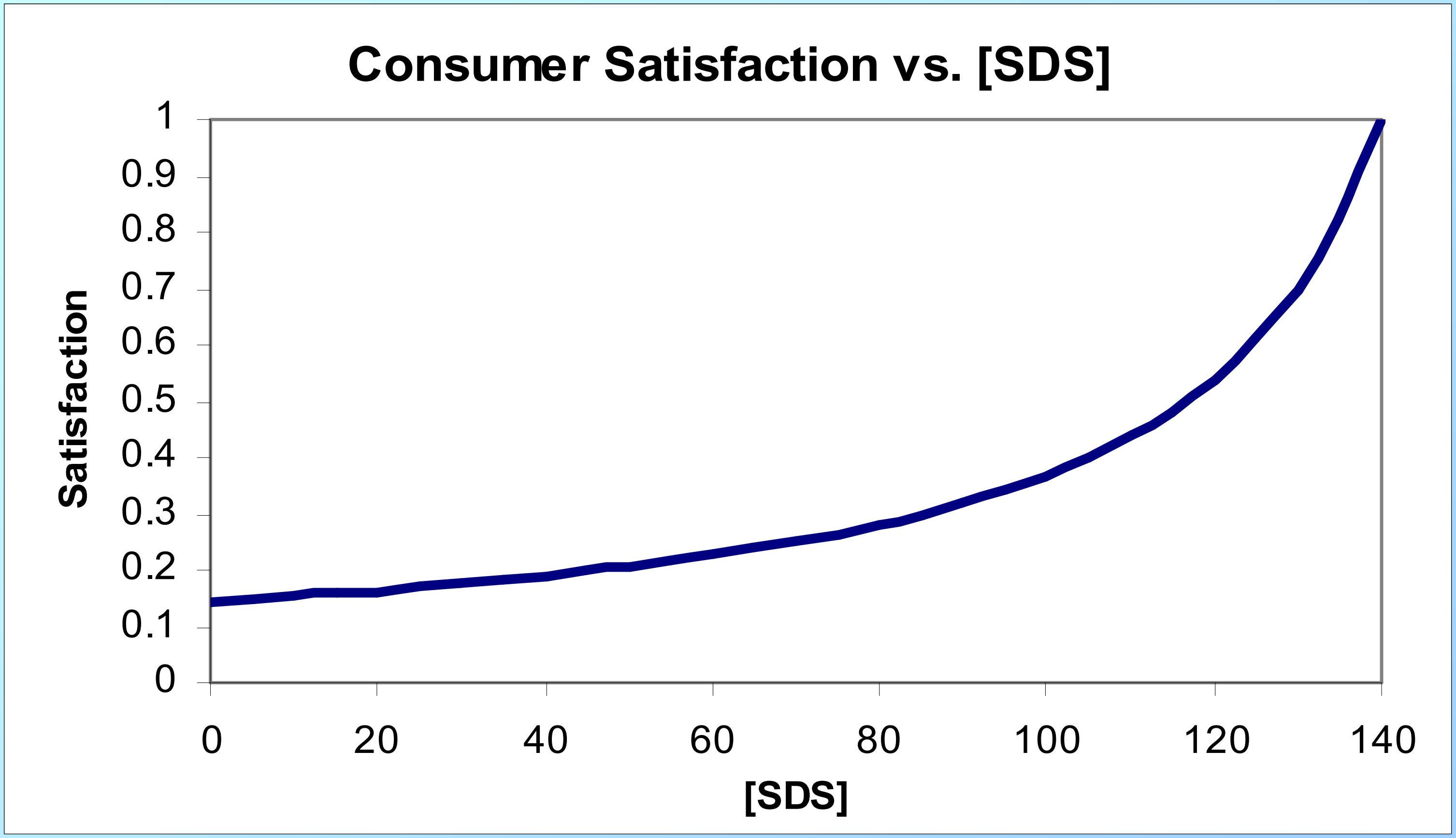
- Resolution
  - Sodium dodecyl sulfate decreases the effects of bilirubin, thereby reducing the likelihood for false negative results

Negative Interference



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

Negative Interference



Satisfaction increases with increasing SDS concentration

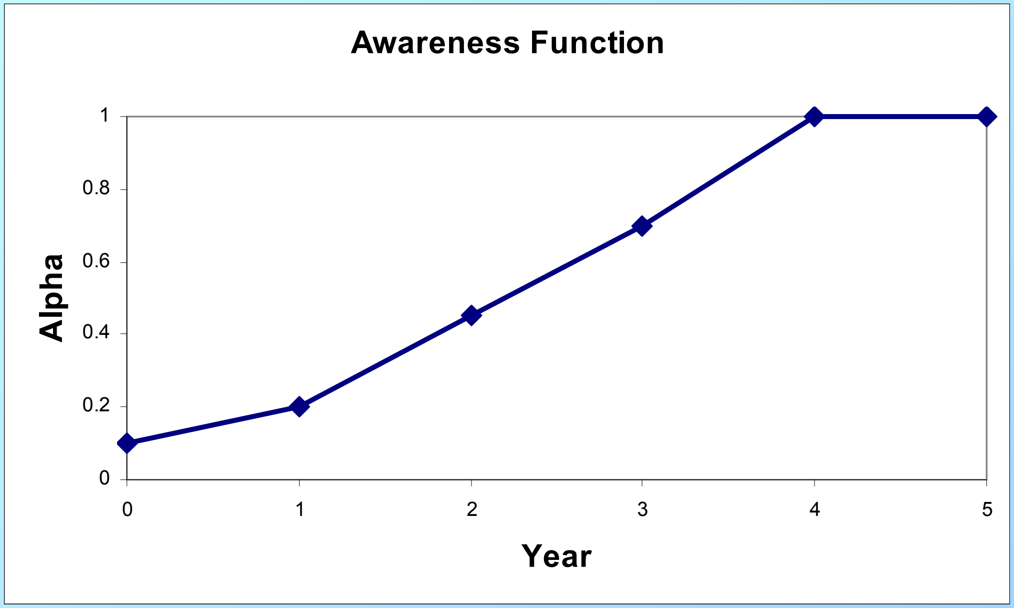
Pricing and Demand Model

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

- p_1: new product price
- d_1: new product demand
- α: describes consumer knowledge of new product
- β: describes consumer preference for new product
- Y: total consumer budget
- p_2: existing product price (serum test – $10/test)
- ρ: constant of 0.75

Awareness Function

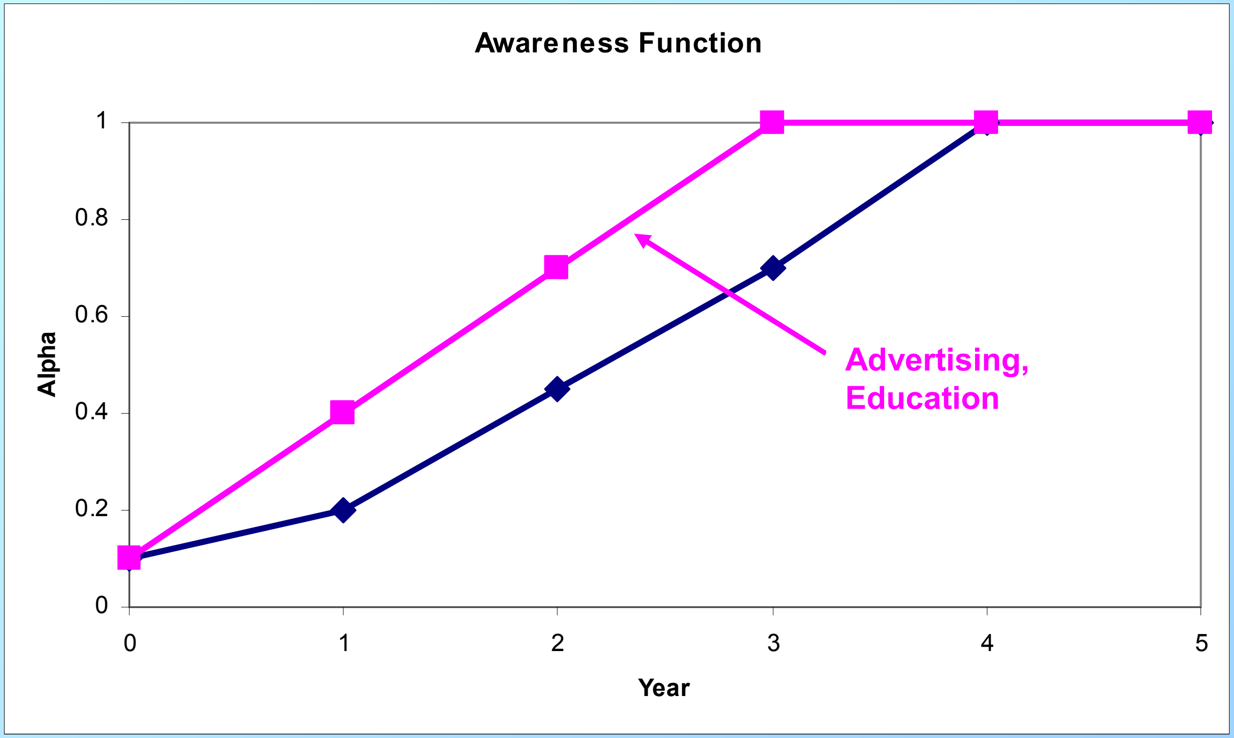
$$\Phi(d_1) = p_1 d_1 - \left(\frac{\alpha}{\beta}\right)^{\rho} p_1 \left[\frac{Y - p_1 d_1}{p_2}\right]^{1-\rho} d_1^{\rho} = 0$$



- Awareness (α) is a function of consumer awareness of the product
- Awareness increases with time to a value of 1, indicating total awareness

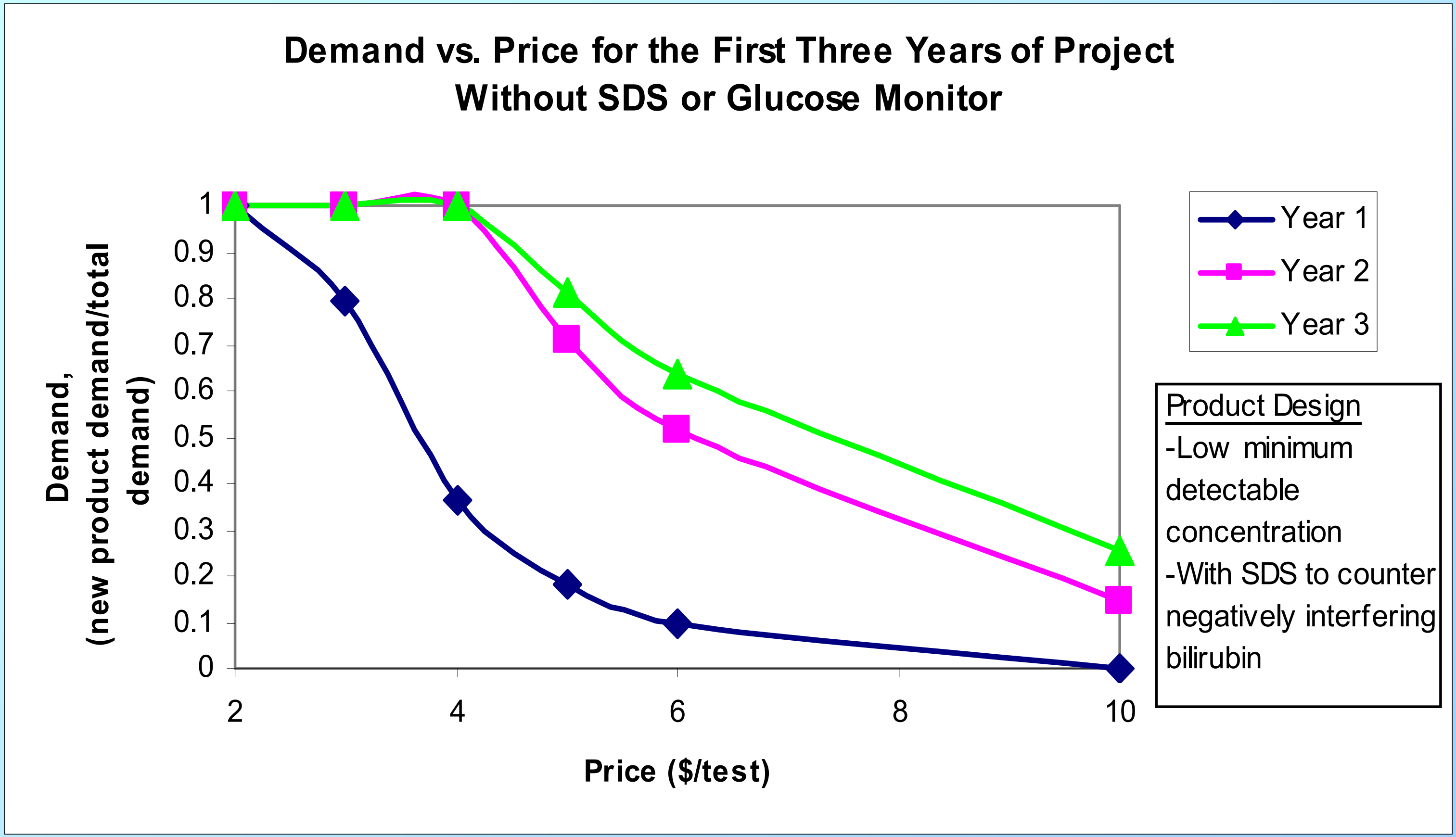
Advertising and Education

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



Advertising and education can increase awareness

Influence of Awareness



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

Beta Function

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

$$\beta = \frac{H_2}{H_1}$$

H_1: Consumer satisfaction for the new product
H_2: Consumer satisfaction for the existing product

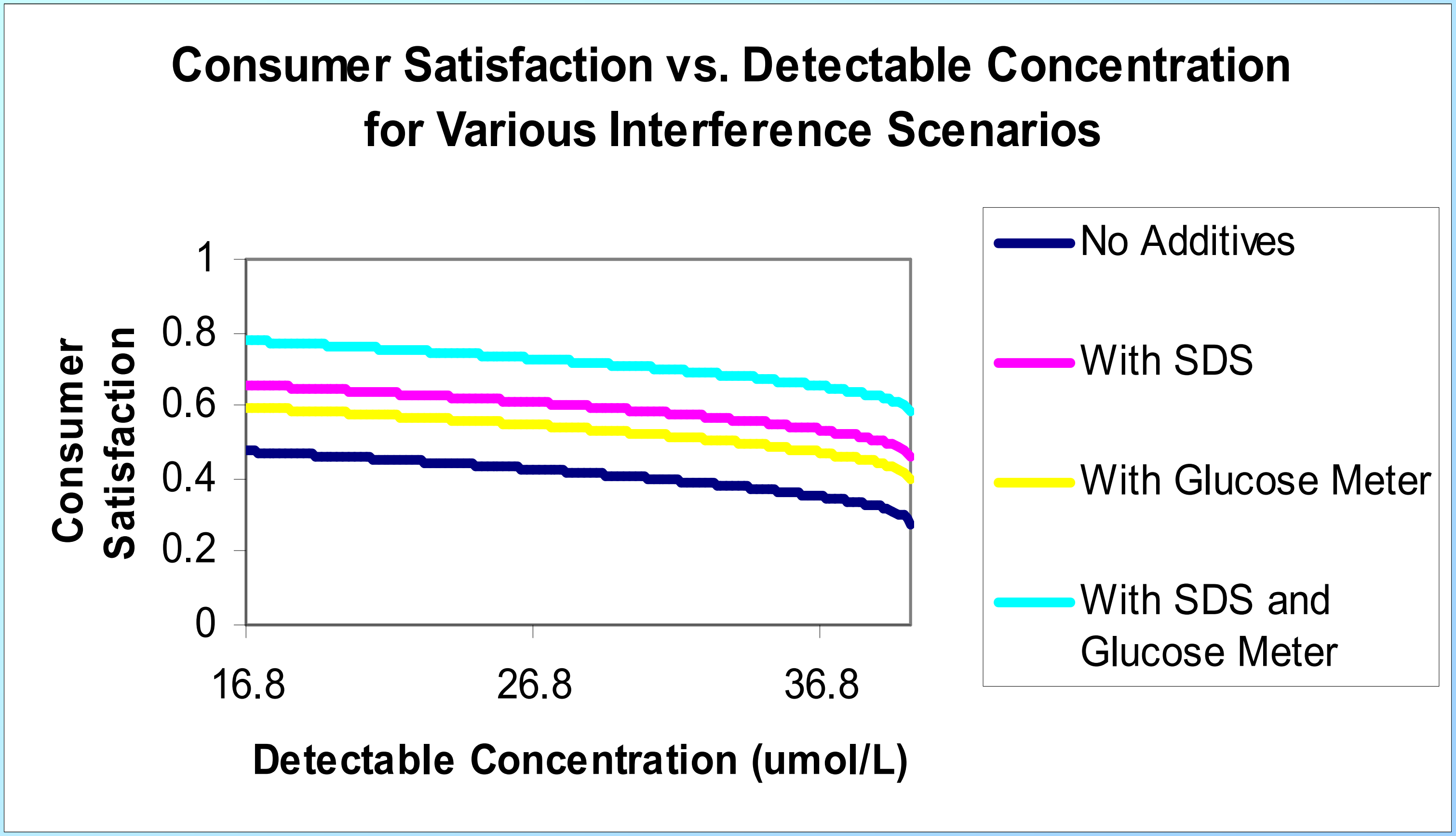
-Lower beta values indicate a more appealing product

Beta does not factor in price

Product Design

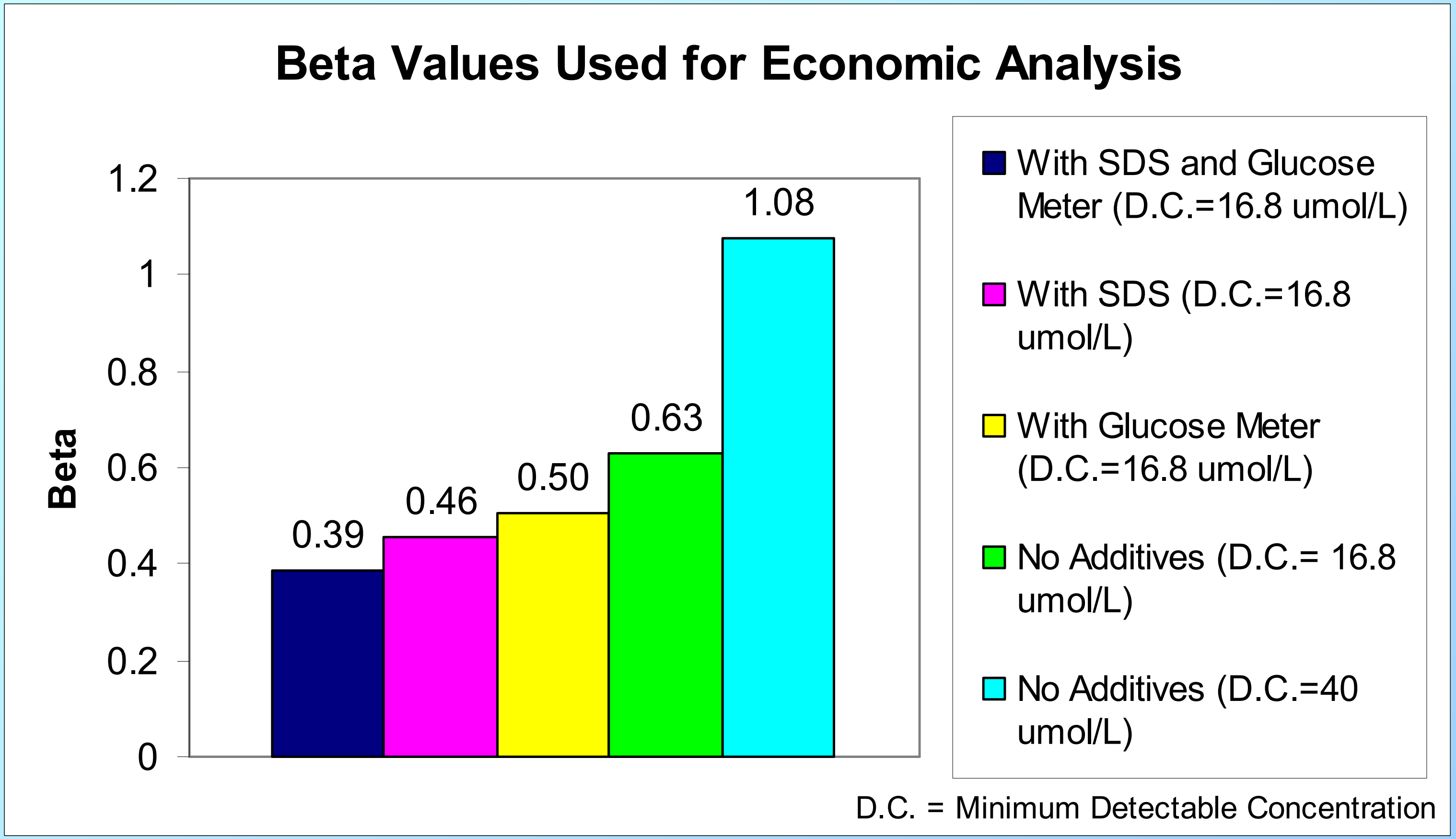
- *Minimum Detectable Concentration*
  - Low
    - 16.8 umol/L
    - Stages 2, 3, 4, and 5
    - Spectrophotometer
    - Includes Standards
  - High
    - 40 umol/L
    - Stages 4 and 5
    - Determined Visually
    - Standards Unecessary
- *Inclusion of Anti-Interference Components*
  - Option 1: No 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



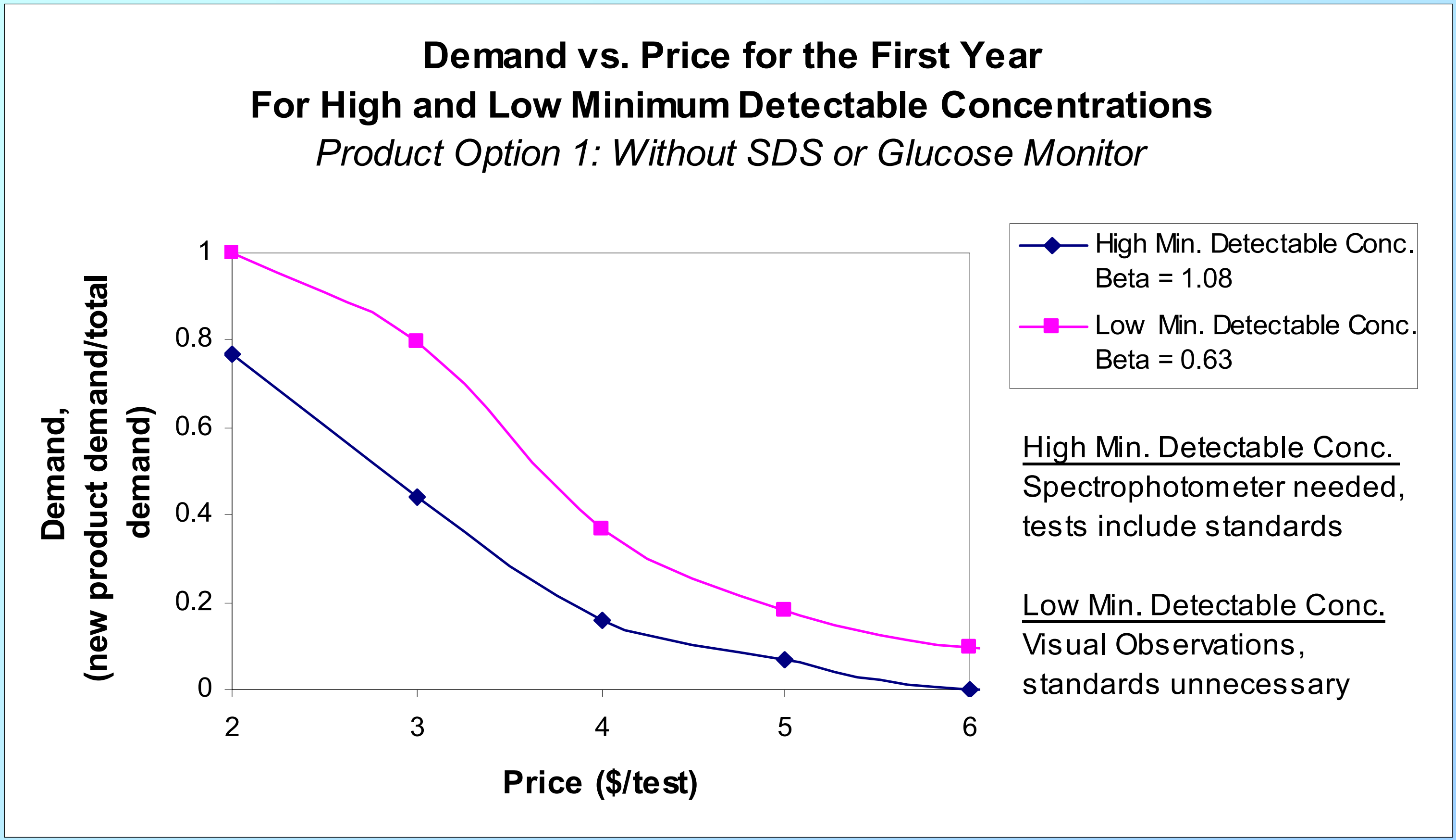
Satisfaction is higher for options including additives to counteract interference

Consumer Preference



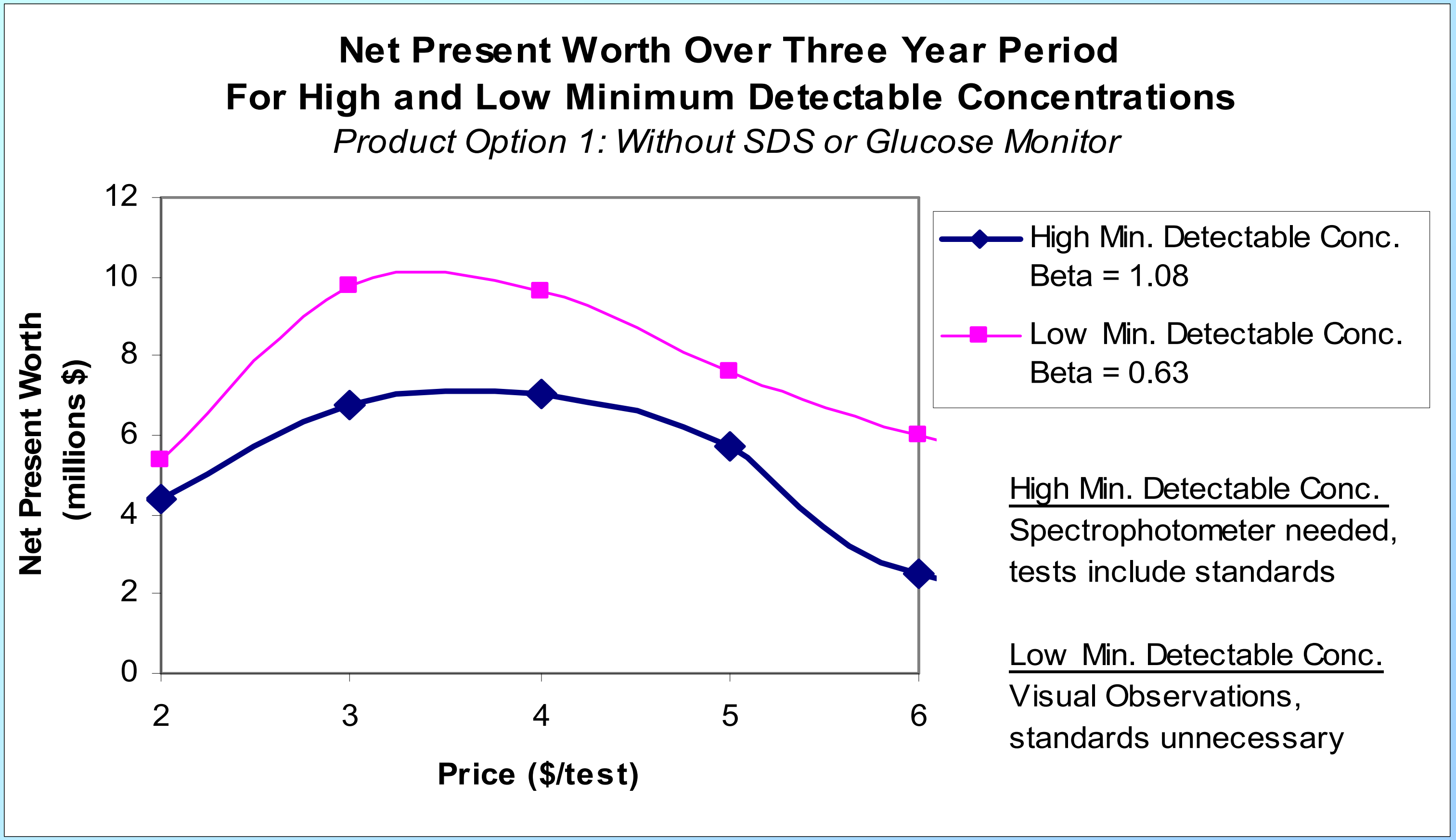
$$\Phi(d_1) = p_1 d_1 - \left(\frac{\alpha}{\beta}\right)^{\rho} p_1 \left[\frac{Y - p_1 d_1}{p_2}\right]^{1-\rho} d_1^{\rho} = 0$$

Price and Demand



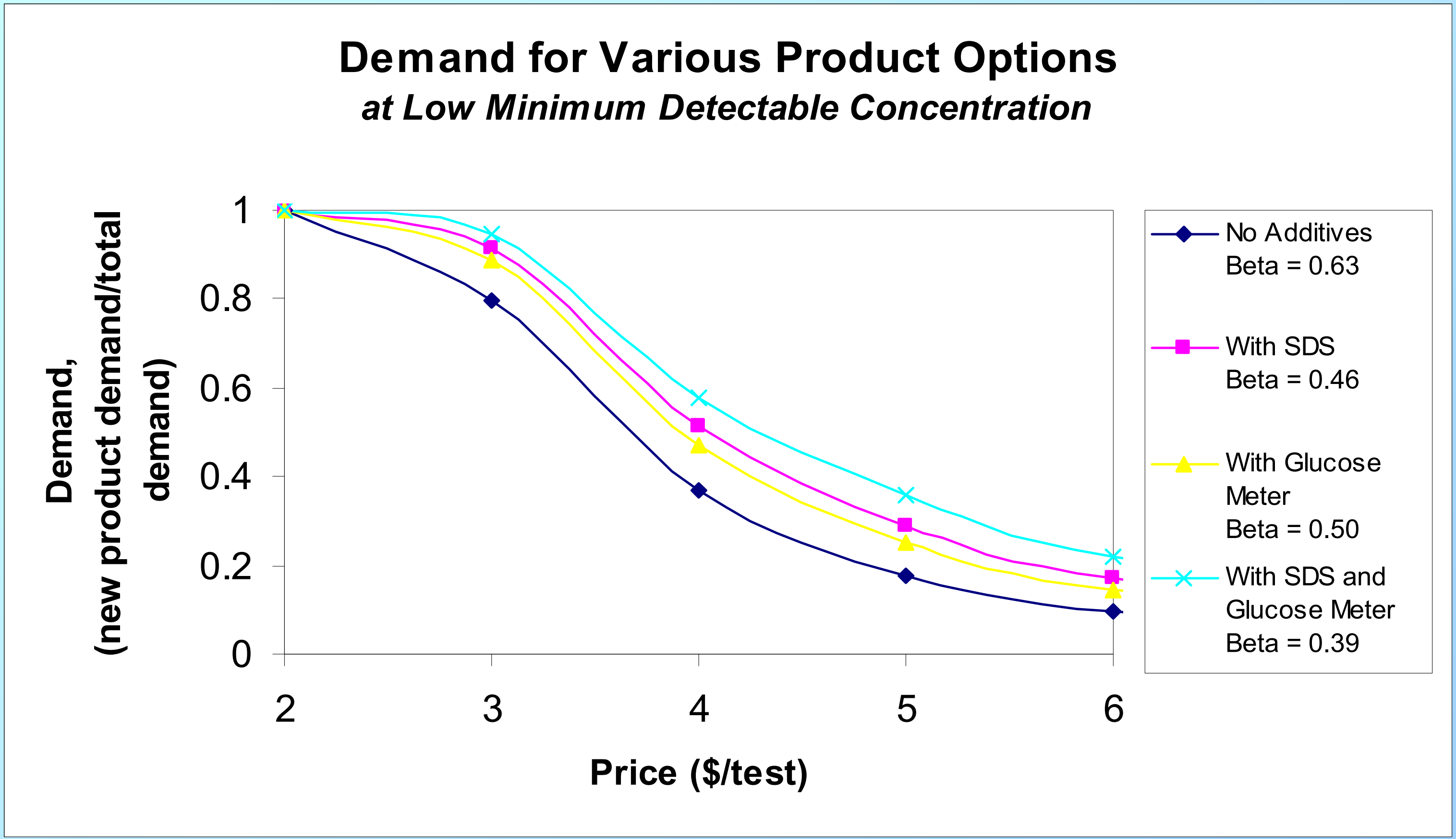
The demand is higher for the test with a lower minimum detectable concentration

NPW for Different Sensitivities



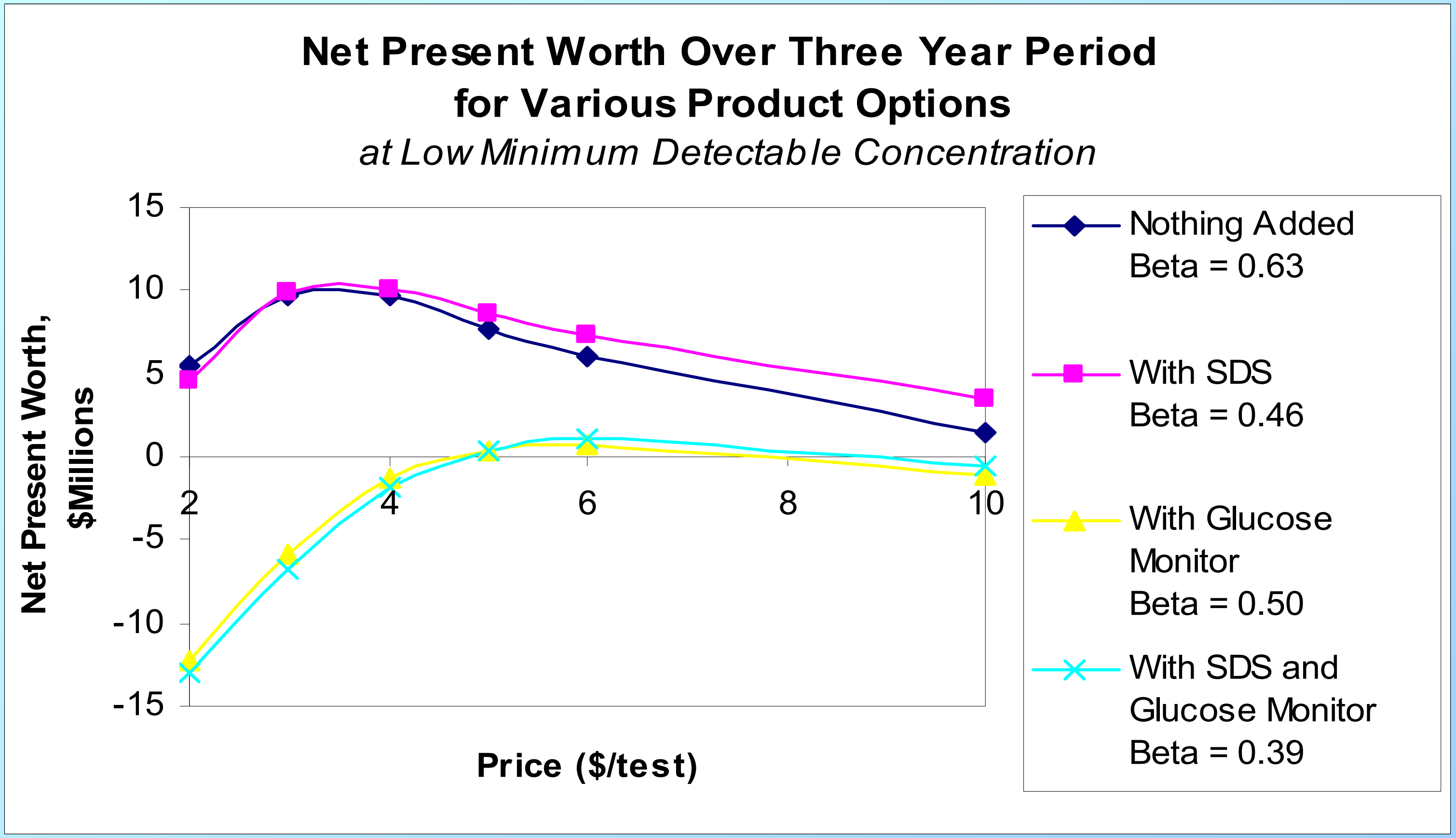
The test able to detect low concentrations was more profitable despite added product cost

Price and Demand



Demand versus price curves were created using the pricing and demand model for the four interference scenarios

Net Present Worth



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

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