Bacteria in Semen
Background:

- The concept of detecting and identifying bacteria by ribosomal RNA gene sequences is about 15 years old.
- Although limited, the application of this approach to clinical specimens has revealed that most (greater than 95%) human pathogens have never been identified by laboratory culture methods.
- A well known example of this is Helicobacter pylori which was detected by molecular biology before it could be cultured.
Why ribosomal RNA (rRNA) genes?

- All living organisms use ribosomes to synthesize proteins.
- Ribosomes have two subunits, designated by their speed of pelleting in a centrifuge:
  - 30S and 50S for bacteria -- combine to form 70S
  - 40S and 60S for mammalian cells -- combine to form 80S
- Each subunit contains both RNA and protein folded together in a very specific conformation
  - The 30S subunit contains 16S rRNA
  - The 50S subunit contains 23S and 5S rRNAs
- Some, but not all, regions of bacterial rRNAs share homology with mammalian rRNAs.
Schematic of role of ribosomes in protein synthesis:
E. Coli 16S rRNA schematic:
Ways to detect bacterial rRNA genes:

- In situ hybridization
- Amplification by Polymerase Chain Reaction (PCR)
  - Forward Primer: AACTGGG...
  - Reverse Primer: AGGAGGG...
- Products identified by
  - Southern Blot analysis
  - Direct gene sequencing
E. Coli 16S rRNA amplified:
Current Method

• Non-sperm semen DNA is isolated with a commercial column
• Start with a total semen volume of 10 to 16 microliters (approximately one loop-full)
• Subjected to 30 cycles of PCR under conditions that will detect on the order of 1000 target genes, equivalent to on the order of 300 bacteria
• Approximately equivalent to 300 colonies of bacteria on a culture plate
Current Method (cont’d)

- PCR products are electrophoresed through an agarose gel, stained with fluorescent dye (ethidium bromide) and visualized with uv light box.

Agarose gel of 18 semen specimens, 13 positive for bacteria.
Current Method (cont’d)

• PCR products from positive reaction tubes are purified through a commercial column.
• The Forward Primer is added to a small aliquot of purified products and mailed to the gene sequencing laboratory.
• The sequences are emailed back to Bryan
• Bryan edits them in software “Sequencher” to eliminate ambiguous bases and other problems.
Current Method (cont’d)

• The edited sequences are then
  • Submitted to BLAST search for identification
  • Compared with other sequences from semen specimens

Important: this method avoids cloning

Sponsored by Bedford Research Foundation
History:

- Two reports appeared in 1996:
  - One from Keith Jarvi’s lab in Toronto
    - 30 infertility semen specimen
      - 8 positive by routine bacterial culture
      - 20 positive by PCR
      - PCR products cloned and sequenced
  - Most organisms not identified in GenBank
  - Found several species of Peptostreptococcus, Streptococcus and Cornybacterium
History (cont’d):

• Second 1996 report from John Krieger’s lab at the U of W:
  – Biopsy study of 135 men with chronic prostatitis
  – 77% of biopsies were positive for bacterial DNA
  – Cloned products from 10 patients had no matches in GenBank
  – Only one third of men with bacteria-positive biopsies exhibited leukocytes in expressed prostatic secretions
History (cont’d):

- Two subsequent biopsy studies from Krieger lab in 1998 and 2000:
  - Bacteria species still not in GenBank, but some related to Staphylococcus epidermis
  - Only 20% of cancer biopsies (107 men) positive for bacteria sequences
  - These two studies used to define two groups of bacteria, “prostate A” and “prostate B”
History (cont’d):

- Another biopsy study in 2000 by Anthony Schaeffer’s lab:
  - Detected no bacterial gene sequences from 18 organ donors biopsied under sterile conditions
  - Two patients with BPH had bacteria-positive biopsies
  - Six of seven patients with prostate cancer had bacteria-positive biopsies

Concluded no “normal flora” in the prostate
History (cont’d):

• A study in 1999 of expressed prostatic secretions (EPS) by Norman Pace’s lab:
  – 17 men with chronic, refractory prostatitis
    • 8 EPS positive for bacteria by culture
    • 11 positive for bacteria by PCR
    • 4 positive men did not respond to antibiotic therapy
  – 8 controls with no prostatitis symptoms
    • 6 positive for bacteria by PCR
  – Prostatitis specimens many more types of bacteria and usually included Cornybacteria
Where we are:

- Conducting three series of patient specimens:
  - Infertility
  - HIV infected
  - Prostatitis

- Results to date only from Infertility series
Where we are:

• Data to date from 18 semen specimens from 15 men
  – 12 (67%) are positive for bacteria
  – Only four positively identified in GenBank
    • Staphylococcus (2), Lactobacillus (1), Cornybacacterium (1)
    • Some maybe related to Peptostreptococcus
    • Some not identified because a mixture of organisms
    • No E. coli detected
  – No correlation with semen leukocyte count
### SEMEN BACTERIA EXPERIMENTS

#### Total Numbers (millions)

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Where we are (cont’d):

Our data basically agree with previous studies

Multiple specimens from two men:
  – Two positive, third undetectable after antibiotic therapy
  – One positive, second negative after therapy
  – Both a mix of bacteria
What we intend to do:

• Continue to study prostatitis patients
• Develop a faster way to identify the bacteria
  – Gene chip technology
  – Need to know identity of organisms
• Begin to study prostate cancer patients
  – Method easily adapted to prostate cancer markers