


# Technology for the production of protein biopharmaceuticals

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Innovation. Experience. Performance.

# Selected topics

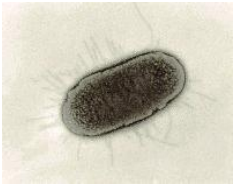
- Expression of recombinant protein biopharmaceuticals
- Acceleration of  microbial process development
- Manufacturing capacity: shortage or excess?
- Developments that impact manufacturing capacity and throughput
- Emerging alternatives to mammalian cell culture
- Yeast systems for the production of monoclonal antibodies (mAb)
- Conjugation of mAb



mAb: monoclonal antibody

# Biopharmaceutical manufacturing: expression systems

- Recombinant protein biopharmaceuticals expressed in bacteria, yeast, insect and mammalian cells
- Emergence of transgenic expression systems : goat, cow, corn, tobacco, duckweed



## Biopharmaceutical manufacturing (clinical + commercial)

Cell culture : 50-60%

Microbial : 40%

Other : <5%

## Expression systems encountered by CMO

*Escherichia coli* : 60%

*Pichia pastoris* : 35 %

*Saccharomyces cerevisiae*

*Hansenula polymorpha*

*Bacillus subtilis*

*Aeromonas salmonicida*

## Microbial expression still a major opportunity

Dominance of *Escherichia coli*

Strong interest in *Pichia pastoris*

Other expression systems available but little market penetration (yet)



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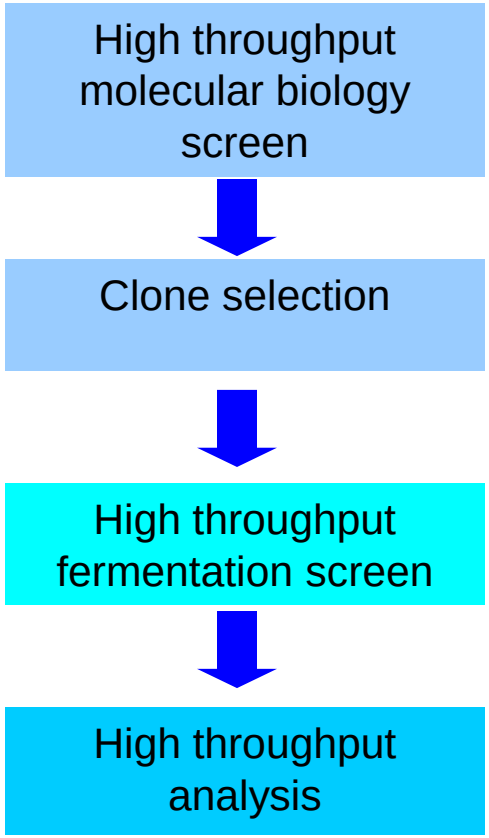
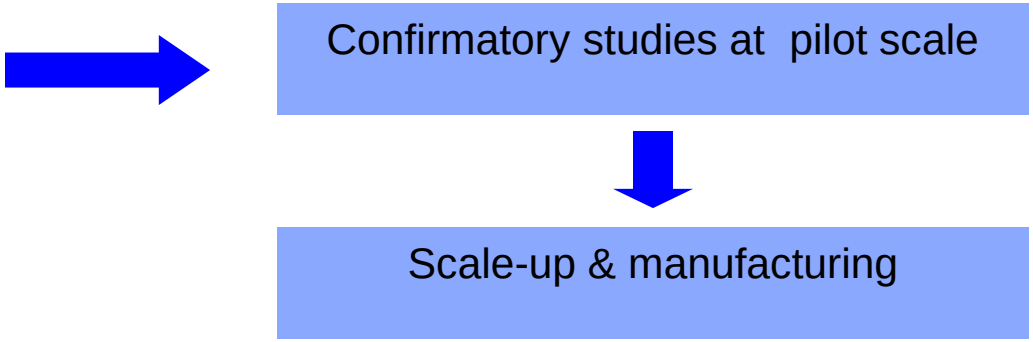
# Accelerating process optimisation of microbial expression systems




Acceleration of microbial process development through new technologies

- Multi-factorial studies
- Identification of poor performance and risk factors
- Selection of high expression permutations



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# Control of expression: future developments

- High throughput (miniature) fermentation
- “Fermentation by design”: design of experiments approach for optimisation
- Map boundaries of fermentation control parameters
- Integrate high throughput analysis to compliment output of fermentation systems

(Image courtesy of BioProcessors Corp.)

Rapid screening of full factorial matrices

Reduce the number of permutations for screening at bench-scale

Predictive tool



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# Manufacturing capacity: shortage or excess?

- Various models to address # products, market demand and manufacturing capacity
- Predictions of capacity shortage, excess and cross-over scenarios
- Technology to alleviate volumetric constraints

CHO cell culture : 1-4g/L expression in stirred tank culture

2 - 5 Years

CHO cell culture : 5-10g/L expression

Reduce in process volume

$ / COG

Greater productivity

Purification becomes production bottleneck?



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# Scales of operation

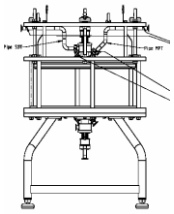
## Upstream processing

- Current scenario of large scale cell culture at 10 000L - 20 000L scale of operations (multiple trains at manufacturing facilities)
- Impact of process development on productivity
- Reduction in process volume by 2-5 factor?

## Downstream processing

- Column volume maximum = 500L (2-3m in diameter)
- If 20g/L, then batch size for each cycle in purification = 10kg
- However, if 20 000L @ 5g/L = 100kg
- Requirement for high capacity and high throughput functional chemistries

# Downstream processing: developments



## Column chromatography

- Improvements to existing functional chemistries (base stable protein A affinity resins, high capacity/flow ion exchange media, flourohydroxyapatite)
- Increased capacity and solids rejection for capture chromatography resins
- Improved hardware for automated packing, sanitary operation

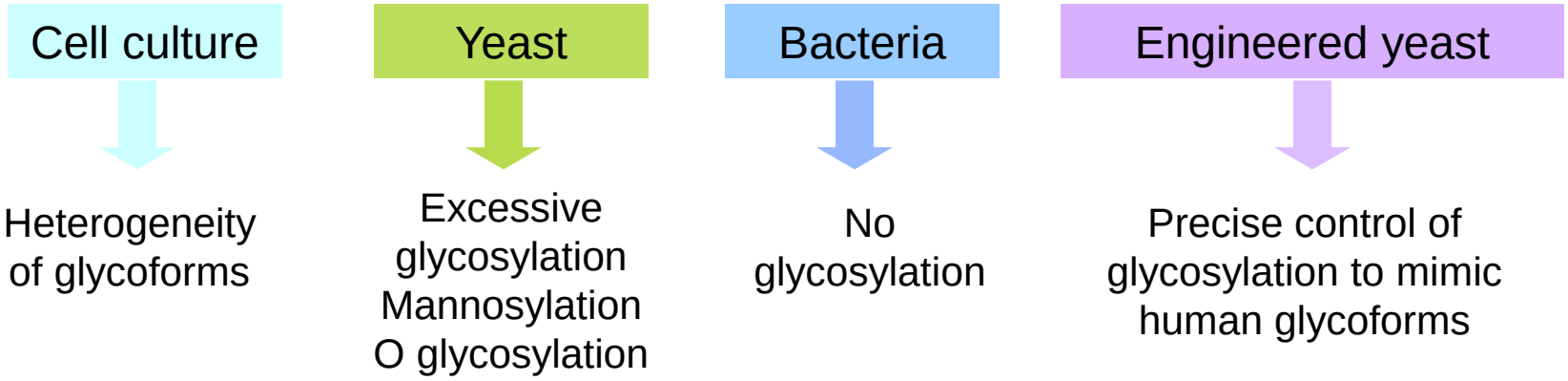
## Membrane based unit operations

- Charged membranes: Efficient reduction in endotoxin and DNA contamination
- Single-use membrane assemblies (pre-fabricated disposable units)
- Improvements in solids handling/flux characteristics

# Emerging alternatives to mammalian cell culture

- Mammalian cell culture market dominated by **monoclonal antibodies** (mAb)
- 18 approved mAb (FDA/EMEA)
- Antibody fragments may be expressed in bacterial expression systems
- Complexity of full antibody structure and function requires efficient assembly and post-translational modifications (glycosylation)
- Human-like glycosylation patterns desirable
- Yeast systems : Emergence of yeast systems to express fully assembled structures with core or human-like glycosylation patterns.

# Control of expression: emerging technology




- Engineered yeast systems (*Pichia pastoris*) may offer high precision of control of glycoforms
- Benefits:
  - Therapeutic advantage (lower dose regime) due to minimum heterogeneity
  - High expression levels / attractive COG
  - Established production scenarios



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# Conjugation of mAb

- Emerging niche market for targeted therapies
- Covalent linkage of mAb with effector (low molecular weight) molecule
- Mode of operation : Selectivity of mAb for target binding followed by cytotoxic activity of effector.
- Increased clinical efficacy against target cells
- Aqueous based coupling chemistries (hydrazone, disulphide, peptide)
- Release of cytotoxic effector mediated via intracellular environmental events : Hydrazone (pH decrease within lysosomes), disulphide (reduction within cytoplasm), peptide (enzymatic hydrolysis)
- Requirements : Experience of protein and small molecule chemistry together with infrastructure for handling of cytotoxic effector

# Opportunities for Cambrex

- GMP asset base is well placed to exploit increases in productivity of microbial systems
- Expertise in the development of manufacturing processes based on yeast expression systems to meet demand for new generation of glycosylated proteins (mAbs)
- Cambrex pharma and biopharma capabilities ideal for protein conjugate technologies and market supply
- Integrated services for full supply chain service (gene to vial)
- Development of collaborative relationships with chromatography resin and membrane manufacturers (“beta” test site): Leading edge technologies capable of implementation in a GMP manufacturing environment

# Innovations in Research Products

Markus Hunkeler

# Cambrex Research Products

Molecular Biology

- Nucleic Acid Electrophoresis
- Protein Electrophoresis

Cell biology

- Primary Cell Systems
- Conditionally Immortalized Cells

Bioassays

- Cell Health and Function
- HTS

Cell Culture

- Media and Sera

# Nucleic Acid Electrophoresis Market

- Electrophoresis market: ~$120M (US)
  - Protein
  - Nucleic Acid
- Three key players
  - Invitrogen
  - Biorad
  - Cambrex
- 60,000 researchers perform nucleic acid electrophoresis (US)
  - ~4-5% market growth
  - Precast Gels – 15-20% growth
  - Market drivers toward precast gels
    - PCR
    - Speed
    - Convenience

# Nucleic Acid Electrophoresis Market

- Cambrex is the market leader in nucleic acid electrophoresis
  - Agarose and Precast Agarose Gels
  - Markers and Ladders
  - Buffers and Stains

- To capitalize on the growing need for speed and convenience, developed an integrated system:
  - Gel, Buffer, Stain, Visualization

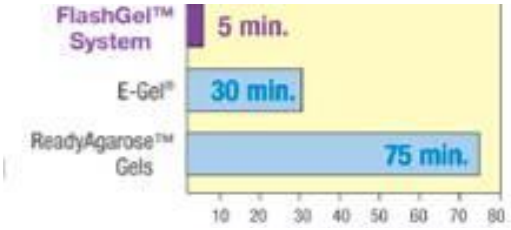
- Result:
  - 5-Minute Experiment

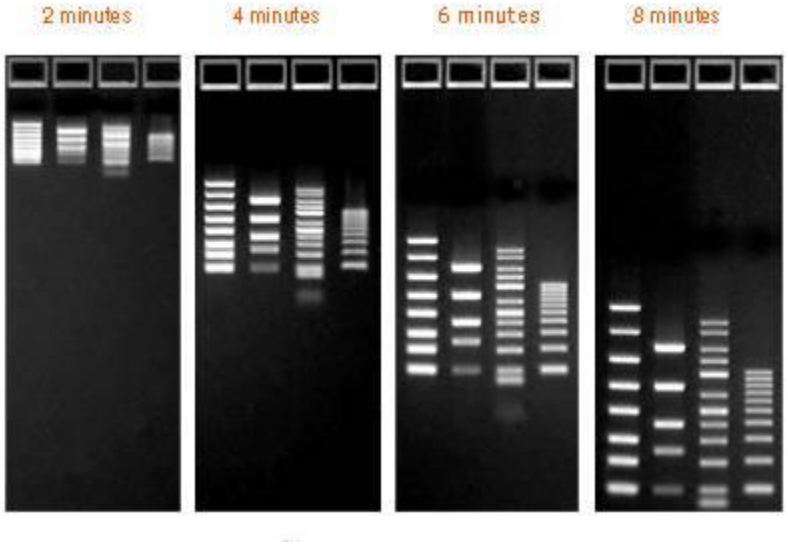
# FlashGel™ System

New in 2005

- Launched September, 2005
- The fastest way to separate DNA
  - Results in 2 – 7 minutes
- Real-time analysis - watch DNA migration as it happens
- Eliminate UV light-”Dark Reader” technology
- Proprietary fluorescent dye – increased sensitivity
- Proprietary agarose blend – “One size fits all”

***Results in 5 minute***



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# FlashGel™ System

- FlashGel™ Cassettes require no prep time
  - Precast, prestained gels and buffer

**FlashGel Dock™**

- The FlashGel™ Dock is an electrophoresis apparatus with build in transilluminator for separation and detection

**I will link the video here**



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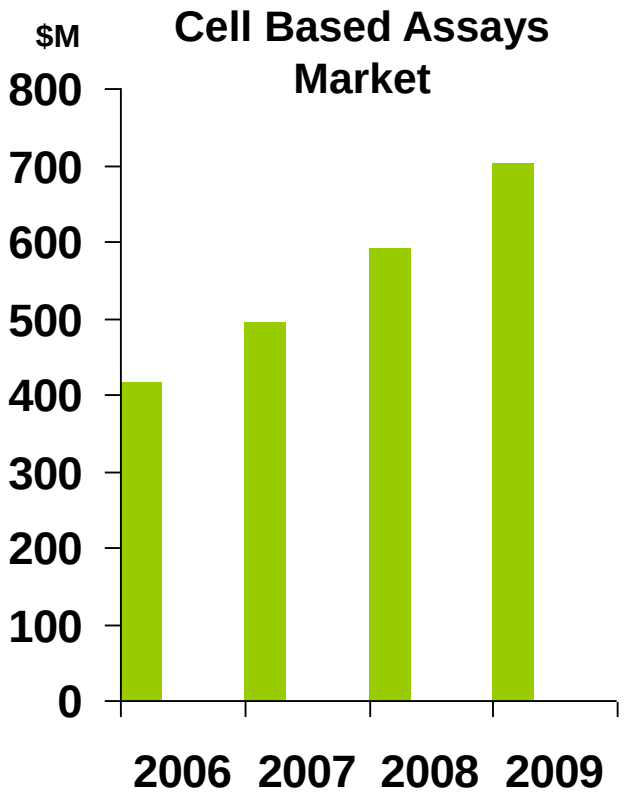
# Cell Based Assays for HTS

- Researchers evaluate a large number of compounds for efficacy and toxicity
- Over 60% of drug candidates fail because of problems with ADME and toxicology
- Tools are needed to facilitate HTS screening earlier in the drug discovery process
  - Eliminate poor drug candidates early
  - Focus on more promising candidates
- Cell based assays offer the potential to screen out compound failure earlier in the discovery and development life cycle.






**HTS: High Throughput Screening**

# Cell Based Assays for HTS

- Cell based platforms offer improved biological relevance
  - Primary cells offer the highest level of biological relevance but suffer from small lot sizes and donor variability
  - Cell lines (immortalized cells) generate large homogeneous cell populations but do not behave like normal cells

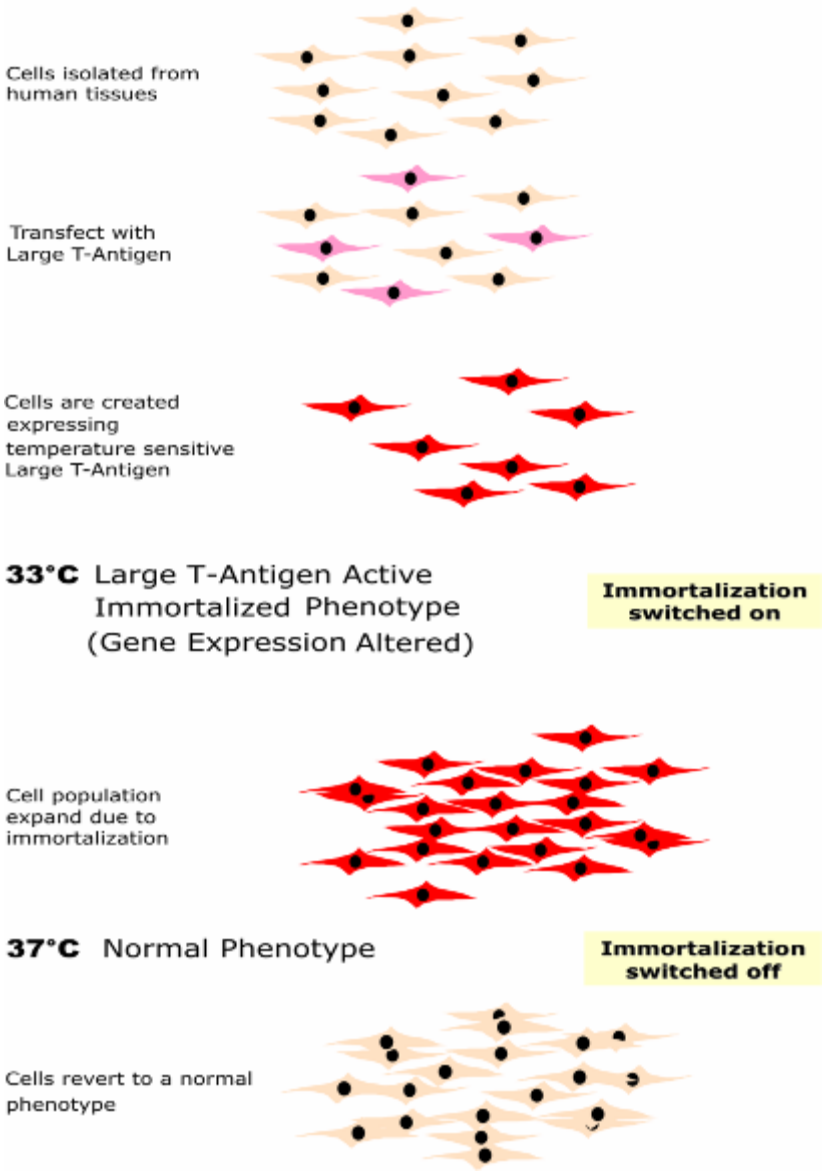
- ***Conditional Immortalization*** allows production of high volumes of uniform “primary-like” cell populations

- Cambrex has assembled unique technology to enable high throughput cell based screening
  - Primary cell expertise
  - Multiple licenses

- Addresses the unmet need
  - Higher biological relevance
  - High throughput

# Clonetics® conditionally immortalized human cells

New in 2005




- Incorporates licensed telomerase technology for immortality
- Incorporates temperature sensitive switch for proliferation
- By changing the culture temperature the cells stop dividing, allowing for differentiation and expression of normal function and phenotype
- Results in a large supply of differentiated cells of the same genotype that can be used as models for functional cell-based assays and long-term gene expression studies



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# Clonetics® conditionally immortalized human cells

- Conditionally Immortalized human skeletal muscle cells derived from normal human skeletal muscle
- Differentiate into well developed myotubes that have morphological characteristics of differentiated muscle and phenotypic characteristics of muscle including creatine kinase activity and insulin induced glycogen synthesis

# Clonetics® conditionally immortalized human cells

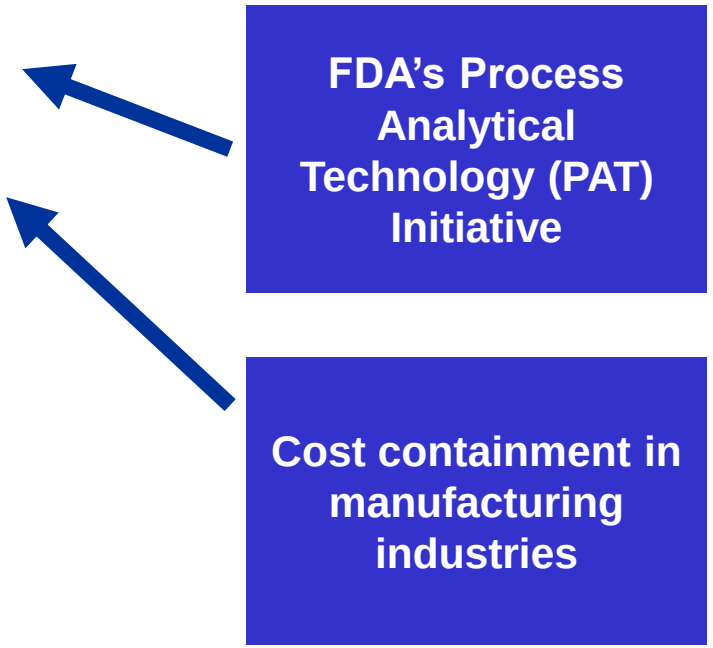
- Launched conditionally immortalized skeletal muscle cells - March 2005
- Conditionally immortalized human adipocytes in Q4, 2005
- Plans to launch another two cell lines each year
  - Intestinal epithelial cells
  - Cardiomyocytes
  - Large Artery endothelial cells
  - Hepatocytes
- Licensing programs
  - Licensed skeletal muscle cells to Chugai Pharmaceuticals
  - Several other pharma/biotech in active negotiations

# Rapid microbial detection

Shawn Cavanagh

# Rapid microbial detection (RMD)

- Focused on developing rapid testing technologies for the pharmaceutical, medical and other consumer industries
  - Endotoxin detection
  - MicroAlert® TVO
  - MycoAlert®



# Endotoxin detection

- Endotoxins are pyrogens that may cause fever, shock or even death if high levels are introduced into the body
- Products routinely tested in the medical device, pharmaceutical, dialysis and bioresearch industries
- Market size ~ $60 – 70 million and growing 3 – 4%.
- Growth opportunities in automation

# Cambrex ‘firsts’ in endotoxin detection

- Providing the total solution
- In quality and compliance
- Innovation
  - With quantitative chromogenic LAL
  - 21 CFR P11 compliant software
  - To commercialize a recombinant endotoxin activated assay (PyroGene®)
  - To develop an on-line endotoxin method for WFI

# MicroAlert® TVO

- Measures total viable organisms (TVO) in less than 5 hours rather than days or weeks
- Differentiated, high-tech solution with an expandable platform for
  - Environmental monitoring
  - Bioburden testing
  - Preservative efficacy testing
  - Water testing
  - Sterility testing
  - Food
- Reduces costs by quickly reducing hold times

**The End of an era?**



TVO: total viable organisms

# Market Size and Growth Rates

| $ millions | Worldwide Total Micro. Testing | Total TVO | TVO Rapid Methods | Total Micro Growth | Rapid Methods Growth |
|---|---|---|---|---|---|
| Pharma | 666.3 | 399.8 | 40.0 | 7% | 10% |
| Personal Care | 485.9 | 391.5 | 29.2 | 7% | 10% |
| Food/ beverage | 1200.0 | 960.0 | 48.0 | 4% | 7% |
| Total | 2352.2 | 1651.3 | 117.1 | | |



Source: Overall market size from Strategic Consulting.

# MicroAlert®-TVO

- Acquired technology in Q4 2004
  - Real-time PCR based methodology
- Beta tests to start in Q1 2006
  - Time to Result of < 5 hours
  - System throughput of 64 samples per shift
  - Applications testing with customer samples will form part of the beta trials
- High level of interest from large pharma, biopharma and consumer products companies
- First sales expected mid-2006

# MycoAlert® mycoplasma detection assay

- Mycoplasma detection in twenty minutes
  - One of our most successful research products
- Validated assay being developed for release testing
  - Current accepted method is 28 days
  - Required sensitivity is 1 CFU
- Collaborating with regulatory agencies for an expedited approval
- Product for validated applications launch in mid-2006

# Rapid microbial detection (RMD)

- Targeting high-value problems and niches that are overlooked
- Providing stepwise innovations to improve test speed, automation and accuracy
- Complete testing solutions
  - Endotoxin detection
  - MicroAlert® TVO testing system
  - MycoAlert® mycoplasma detection system
- Continue to innovate in patient and consumer safety testing

# Cambrex Investment Highlights

- **Well positioned in life sciences and transitioning to specialty pharma**
  - **Leading positions in key segments**
  - **Building on existing technologies and capabilities**
  - **Moving towards higher growth opportunities**
- **Above average growth potential**
  - **Favorable demographics and regulatory environment for therapeutics**
  - **New technologies for drug discovery and healthcare offer growth opportunities**
- **Innovative products and services**
  - **Proprietary products and technologies**
  - **Development & manufacturing expertise**
  - **Experience making licensed therapeutics**
  - **Sales and marketing services**
- **Diversified products and services base**
  - **Multiple routes to success**
  - **Strong financial position**

# Q&A

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Innovation. Experience. Performance.