Celsius Rapid Detection:
AKuScreen™
Adenylate Kinase-amplified bioluminescence
Discussion Points

- The Cost Savings from Rapid Detection
  - How do RMMs drive cost savings?
- The Celsis Rapid Detection System
  - How does the AKuScreen assay work?
  - Why is Celsis the right choice?
- System implementation
  - Ease-of-use, ease-of-implementation considerations
The Cost Savings of Rapid Detection

**Traditional Micro Testing** • Subjective: “The eye”

- **Production impact:**
  - MLT: 3–7 days
  - Sterility: 14–17 days

**Celsis Rapid Detection** • Objective: Instrument-based diagnostic

- **Production impact:**
  - MLT: 24 hours
  - Sterility: 2–7 days

• Have you considered speed-to-market improvements with the implementation of a rapid system?
• What products might benefit from earlier micro-hold release and shorter lead times?
The Cost Savings of Rapid Detection
Work-In-Process Inventory Impact

Reduce production cycle time ➔ Reduce inventory ➔ Savings

Celsius | Rapid Detection
The Cost Savings of Rapid Detection

Work-In-Process Inventory Impact

Reduction in inventory = $650,000

Celsius Method

Traditional Method

Production Cycle (in days)
The Cost Savings of Rapid Detection

View from the Quality Department budget

- Traditional Method
- Celsis Method

Quality Department Direct Costs

Increased costs in Quality Department include reagents and consumables
The Cost Savings of Rapid Detection
A broader perspective

<table>
<thead>
<tr>
<th>Traditional Method</th>
<th>Celsis Method</th>
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<tbody>
<tr>
<td>Quality Department Direct Costs</td>
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<tr>
<td>Investment in Finished Goods</td>
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<tr>
<td>Investment in Safety Stock</td>
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<tr>
<td>Warehouse Space Costs</td>
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<td>Contamination Recovery</td>
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Hidden costs add to true cost per test

Increased costs in Quality Department include reagents and consumables
By reducing microbial testing times, supply chains can save millions by:

- Recovering faster from contamination events
- Reducing inventory requirements
- Improving warehouse utilization
- Overall supply chain efficiency opportunities

- Reducing the cost of operating manufacturing lines
- Improving response / lead times to ship product
The Cost Savings of Rapid Detection
Quantifying the Value

The Celsis Impact Report

- Quantifies financial and environmental impact
- Company-specific inputs
- Company-specific savings
- Thorough, customized financial report

• What other benefits would your organization gain with reduced manufacturing cycle times?
The Celsis Rapid Detection System

Celsis Advance™ luminometer
Celsis Advance.im software
Celsis AKuScreen™ & RapiScreen™ reagents
ATP is a vital component of the metabolic pathway of all living organisms including micro-organisms.

In the presence of the luciferase enzyme, ATP rapidly reacts with luciferin and oxygen to produce a photon of yellow-green light:

$$\text{luciferase} \quad \text{ATP} + \text{O}_2 + \text{luciferin} \quad \text{Mg}^{++} \quad \text{AMP} + \text{CO}_2 + \text{oxyluciferin} + \text{pyrophosphate} + \text{LIGHT}$$

The light signal is detected as RLUs using the Celsis Advance luminometer.

The RLUs directly correlate to the level of ATP which directly correlates to presence of micro-organisms.

The Celsis RapiScreen family of assays are based upon ATP bioluminescence.

The user-validated protocol ensures distinguishable levels of ATP when microorganisms are present.
The sensitivity of an ATP bioluminescence assay can be improved by utilizing adenylate kinase (AK) to generate significantly higher levels of ATP via:

\[
\text{2ADP} \xleftrightarrow{\text{AK}} \text{AMP + ATP}
\]

- The ATP generated by AK can use the ATP bioluminescence reaction.

- *AK can produce in 1 minute approximately 40-fold more ATP than the micro-organism originally contained.*

- *AK is a catalyst for this reaction, and therefore is not consumed during the reaction. Thus, additional ATP will be created as long as ADP is available.*

- Developed and patented by UK Ministry of Defence.

- Celsis holds exclusive rights to the use of AK for product testing in the pharmaceutical, beverage and personal care markets.
ATP is present in all living things – including microorganisms.

The standard ATP bioluminescence assay catalyzes microbial ATP into AMP and light using the enzyme Luciferase.

The light generated is measured using a luminometer.

Luciferase

\[ \text{ATP} + 0_2 + \text{D-Luciferin} \rightarrow \text{AMP} + \text{CO}_2 + \text{Oxyluciferin} + \text{Pyrophosphate} + \text{LIGHT} \]

\[ \text{Mg}^{++} \]
Adenylate Kinase (AK) can be used as a catalyst to convert ADP into ATP.

AK is an enzyme – not depleted by the reaction; therefore can be used to generate almost unlimited amounts of ATP.

Two-stage reaction

- AK + ATP

Gold standard for viability – growth plus Amplification for reduced time-to-results.

How It Works

Celsis AKuScreen reagents use AK to generate more ATP, quickly increasing the glow signal 1000-fold or more.
The Celsis Rapid Detection System
The Technology – AK-amplified Bioluminescence
Celsis AKuScreen™

- Combined principles of microbial growth and biomarker amplification
  - Same targeted detection – viable cells
  - Reduced dependence upon growth for detection

- Results in significant reduction in time-to-detection
  - 18-24 hours for most applications
  - 3-5 days for sterility applications
The Celsis Rapid Detection System
The Technology – AK-amplified Bioluminescence
Celsis AKuScreen™

- Sensitivity enhancement
  - Removed dependence on microbial ATP through generation/amplification cycle
  - Minimized background noise
- Result
  - Significantly higher Signal to Noise (S/N) ratio
  - Increased sensitivity directly relates to a quicker time to detection.

A comparison of S/N ratio between AK-ATP and ATP across positive samples.
The Celsis Rapid Detection System
.im Software

- Advance.im™ software controls the injection of reagents and measures the light generated by reaction
  
  \[ \text{ATP} \rightarrow \text{light} \rightarrow \text{RLU} \]
  
- Any sample that generates an RLU >2x broth baseline is interpreted as positive
  
- In practice, positive samples are >100,000 RLU
The Celsis Rapid Detection System
AKuScreen reagents

Celsis LuminAMP™
- Includes purified ADP converted by adenylate kinase to ATP

Celsis LuminEX™
- Designed to extract microbial ATP from the cell

Celsis LuminATE™
- Luciferase/luciferin in a freeze-dried form

LuminATE Buffer
- For the reconstitution of LuminATE
The Celsis Rapid Detection System

Application

- Pre-screen to Pharmacopeia Microbial Limits test
  - Products testing **negative, pass**.
  - Products testing **positive** are then evaluated per additional testing for enumeration/identification.

- Replaces Pharmacopeia Sterility test*
  - Products testing **negative, pass**.
  - Products testing **positive, fail**.
  - Remaining sample can be used for subsequent investigation.

* Celsis AKuScreen does not exclude a visual inspection for turbidity.
The Celsis Rapid Detection System
The Basic Method – direct inoculation option

A familiar three step process:

Prepare sample  Incubate *(offline)*  Assay

Similarities to traditional qualitative methods
The Celsis Rapid Detection System
Sample preparation, Media selection

- Sample preparation not dictated by method
- Allows for same approach to sample prep as conventional methods
  - Only requirements:
    - Liquid media with low ATP and AK content
    - Product is adequately neutralized
  - Supports organism recovery and growth
    - Sterility - Two growth media (TSB and FTM) are typical
    - Additional or alternate growth media may be explored.
The Celsis Rapid Detection System
Comparison to visual inspection – sterility testing

- Traditional methods are solely dependent upon microbial growth to the point of subjective, visual turbidity

An example where turbidity is a poor indicator of microbial contamination.

Samples 1 & 2 are sterile

Samples 3 & 4 are contaminated
The Celsis Rapid Detection System
Comparison to visual inspection – sterility testing

- Visual inspection is difficult to apply to many non-filterable or non-soluble products.
- The Celsis assay does not require a sub-culture step for samples that render the media turbid.

Gauzes, topical creams, ointments, cosmetic dyes, gels, etc
The Celsis Rapid Detection System
Comparison to visual inspection – sterility testing

- Visual inspection not excluded but not required

- At final time point, pipette 50ul in duplicate, per sample, and assay per Celsis AKuScreen.
  - Objective adjudication and data capture
  - PASS / FAIL

- RLU value in excess of cut off indicates the presence of microbial contamination
The Celsis Rapid Detection System
Why it makes sense

Critical Information
Gives you a fast, decisive ‘yes’ or ‘no’ result:
Rapid release or rapid response

Technical Expertise
Unparalleled experience and technical support

Lab Efficiency
Minimal requirements:
Easy-to-learn, easy-to-use, reduced waste

Broad Range of Applications
Validated on hundreds of products:
if you can test it, we can test it
The Celsis Rapid Detection System

Critical Information

Products test negative: Rapid release
Reduced inventory and cost to manufacture

Products test positive: Rapid response
Reduced contamination costs
The Celsis Rapid Detection System

Robust protocol applicable to many product variations:

- Filterable / Non-filterable
- Soluble / Non-soluble
- High pH / Low pH
- Oil based / Water based
- Pigmented / Non-pigmented
- Preserved / Non-preserved

Examples of Product Types Tested with Celsis
(an abbreviated list)

<table>
<thead>
<tr>
<th>Beauty/Health</th>
<th>Household</th>
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<tbody>
<tr>
<td>Body washes, soaps</td>
<td>Soaps and fabric softeners</td>
</tr>
<tr>
<td>Lotions &amp; creams for face and body</td>
<td>Hard surface cleaners</td>
</tr>
<tr>
<td>Toothpaste, deodorants, gels and mousses</td>
<td>Deodorized products</td>
</tr>
<tr>
<td>Cosmetics: powders, mascaras, eye shadows, etc</td>
<td>Dishwashing detergent: powders, tablets and liquids</td>
</tr>
<tr>
<td>Aerosols, raw materials, wipes</td>
<td>Furniture polish</td>
</tr>
<tr>
<td>OTCs, gelatin caps, vaccines</td>
<td>Household cleaners</td>
</tr>
</tbody>
</table>
System Implementation

3 Lab Efficiency Considerations

People
- Familiar, 3-step protocol is easy-to-learn and easy-to-use

Documentation
- Software provides objective data management and instrument operation and 21 CFR Part 11 compliance capability

Waste Reduction/Sustainability
- Significant reduction in lab waste, energy, water and materials usage

Space Efficiency
- Small foot-print / high-throughput instrument
- 48 x 30 x 57 cm
- 19 x 15 x 22.5 in
System Implementation

Technical Expertise

Technical
- Experienced technical support
- 3 days of on-site training
- Global distributor network

Validation
- IQ / OQ / PQ documentation and validation guides
- In-house validation laboratories

Regulatory
- Drug Master Files
- Technical Reports
System Implementation

4 Technical Expertise

Celsius - Trusted, Experienced

- Global leader in rapid microbial detection systems
- Systems designed exclusively for industry
- Worldwide technical support and distribution
- Broad implementation
  - 100+ million assays
  - 500+ instruments
  - Over 50+ countries

Recipient of numerous industry awards

Supply & Demand Chain Executive

Manufacturing Business Technology

Frost & Sullivan

Frost & Sullivan
The Celsis Rapid Detection System
Some of the world’s leading companies rely on Celsis
The Celsis Rapid Detection System
Some of the world’s leading companies rely on Celsis

- 40 manufacturing facilities
- Customer since 1998
- Contract manufacturers

- 28 manufacturing facilities
- Customer since 1996
- Contract manufacturers

- 37 manufacturing facilities
- Customer since 1994
- Contract manufacturers
Three Questions

1. Why Rapid? Will it reduce my costs?
   Yes - The right rapid method reduces inventories, shortens production cycle times and alerts of contamination faster – the savings are significant

2. Why Celsis? Is Celsis the right system choice?
   Yes - The Celsis system provides critical information on the widest testable product range

3. Can I implement?
   Yes - The Celsis system is easy-to-use, robust and comes with unparalleled worldwide technical expertise and validation support