Sustainable Practices to Reduce Waste & Environmental Impact

Your Ally in Advancing Quality

Kevin L. Williams, May 3rd 2023
Midwest PDA
CURRENT SITUATION

• Governments, regulators, standard setters (USP/EP/JP), and corporations are looking for ways to increase sustainability, supply chain resilience, improve animal welfare and reduce environmental impact.

• What is sustainability?

"Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs."

• Why NOW?

Supply chain resilience + improve animal welfare + reduce environmental impact = Sustainability
CURRENT SITUATION

- **Industry** (has no shortage of) issues:
  - Supply Chain / pandemic recovery
  - Climate change
  - Animal welfare
  - Prices / Inflation / Debt
  - Employment disruptions

- **Theme for us now:** Small steps can make **BIG** improvements toward **SUSTAINABILITY**...
CURRENT SITUATION (BET)

- Massive need for HSC derived LAL in Pharma
- Only US compendial test ...for now
- Global demand growing faster than U.S. demand
- Sourcing Issues: the further from the source of LAL, the more likely supply chain issues are to occur
- Let’s look at RECOMBINANT USAGE in general before looking at rFC more specifically
RECOMBINANT PRODUCTION IS VERY EFFICIENT

1958 > 1982

Not sustainable

More than 50,000 animals were needed to produce just one kilogram of insulin (enough to treat fewer than a thousand diabetic patients for just one year). Because of this, in the early 1970s, there were widespread fears about limited and uncertain supply...

Quickly followed by:
2. Human growth hormone
3. Blood clotting factors
4. Hepatitis B vaccine, an
5. Diagnosis of HIV infection
The disruption of insulin – The first product from precision fermentation, Sept. 3, 2020

https://rethinkdisruption.com/the-disruption-of-insulin/

RECOMBINANT PRODUCTION IS VERY EFFICIENT

Precision fermentation proteins already competitive in medicine, materials, and now food!

Source: RethinkX
RECOMBINANT PRODUCTION = MODERNIZATION

INSULIN CREATED FROM YEAST
Fraction of cost with far greater functionality

Long-acting
Intermediate-acting
Short-acting
Rapid-acting
Etc.

New functionality could not have been achieved using naturally harvested materials.

https://rethinkdisruption.com/the-disruption-of-insulin/
ANIMAL HARVESTED INSULINS RARELY USED

- Cats and Dogs do NOT use animal insulin but use RECOMBINANT human insulin.
- Recombinants are sustainable and “improvable” – manipulation of structure and associated genetic inserts for expression.
RECOMBINANTS TREATING & CURING DISEASE (IMMUNOLOGY)

rDrugs can pull the multitude of levers present on T cells, B cells, etc.

Monoclonals have been fit to block or activate dozens of these targets for cancer and autoimmune disease treatment.
ANOTHER EXAMPLE OF ON-GOING PARADIGM...

Cell-based meat approved in the US for the first time ever

November 21, 2022 / By Taylor Hinds

Earlier this week it was announced that the United States Food and Drug Administration (FDA) has declared UPSIDE Foods’ cultured chicken safe to eat. This approval came in the form of a No Questions List (or GRAS, or Generally Regarded As Safe) designation. The FDA has stated that the product is safe for its intended use, which is a significant milestone for the cell-based meat industry.

https://upsidefoods.com/
**ENDOLISA®**

ENDOLISA® is a new Functionality achieved with recombinant Factor C (rFC)

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**Table 1** | Highest tolerated concentrations of substances for valid LPS spike recovery, a comparison between EndoLISA and LAL assay.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Solvent</th>
<th>EndoLISA</th>
<th>LAL assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer/pH</td>
<td>Acetate (pH 4.0)</td>
<td>100 mM NaCl</td>
<td>50 mM</td>
</tr>
<tr>
<td>Acetate (pH 5.0)</td>
<td>100 mM NaCl</td>
<td>100 mM</td>
<td>12.5 mM</td>
</tr>
<tr>
<td>MES (pH 6.0)</td>
<td>100 mM NaCl</td>
<td>100 mM</td>
<td>5 mM</td>
</tr>
<tr>
<td>Potassium phosphate (pH 7.2)</td>
<td>50 mM</td>
<td>100 mM</td>
<td></td>
</tr>
<tr>
<td>Imidazole (pH 7.4)</td>
<td>Water</td>
<td>500 mM</td>
<td>40 mM</td>
</tr>
<tr>
<td>HEPES (pH 7.5)</td>
<td>100 mM NaCl</td>
<td>100 mM</td>
<td>100 mM</td>
</tr>
<tr>
<td>Sodium borate (pH 9.0)</td>
<td>100 mM NaCl</td>
<td>100 mM</td>
<td>50 mM</td>
</tr>
<tr>
<td>Salt</td>
<td>NaCl</td>
<td>Water</td>
<td>1 M</td>
</tr>
<tr>
<td>KCl</td>
<td>Water</td>
<td>1 M</td>
<td>0.25 M</td>
</tr>
<tr>
<td>Chaotropic agent</td>
<td>Urea</td>
<td>Water</td>
<td>6 M</td>
</tr>
<tr>
<td>Guanidinium chloride</td>
<td>Water</td>
<td>1 M</td>
<td>0.05 M</td>
</tr>
<tr>
<td>Organic solvent</td>
<td>Methanol</td>
<td>Water</td>
<td>20%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Water</td>
<td>30%</td>
<td>0.5%</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>Water</td>
<td>20%</td>
<td>2%</td>
</tr>
<tr>
<td>DMSO</td>
<td>Water</td>
<td>10%</td>
<td>2%</td>
</tr>
<tr>
<td>Detergent</td>
<td>SDS</td>
<td>Water</td>
<td>0.05%</td>
</tr>
<tr>
<td>CTAB</td>
<td>Water</td>
<td>0.004%</td>
<td>0.0001%</td>
</tr>
<tr>
<td>Zwittergent 3-14</td>
<td>Water</td>
<td>0.02%</td>
<td>0.005%</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Water</td>
<td>2%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>Water</td>
<td>0.02%</td>
<td>0.005%</td>
</tr>
<tr>
<td>Chelator</td>
<td>EDTA (pH 8.0)</td>
<td>Water</td>
<td>0.4 mM</td>
</tr>
<tr>
<td>Citrate (pH 7.5)</td>
<td>Water</td>
<td>10 mM</td>
<td>10 mM</td>
</tr>
<tr>
<td>Protease inhibitor</td>
<td>Benzamidine</td>
<td>Water</td>
<td>100 mM</td>
</tr>
<tr>
<td></td>
<td>PMSF</td>
<td>2-Propanol</td>
<td>5 mM</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Rifampicin</td>
<td>Methanol</td>
<td>3.5 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>Ethanol</td>
<td>3.5 mg/ml</td>
</tr>
</tbody>
</table>

*Highest concentration tested.*
U.N. SUSTAINABILITY DEVELOPMENT GOALS

https://sdgs.un.org/goals
U.N. SUSTAINABILITY DEV. GOALS CITED BY PHARMA

Drug Manufacturers are developing strategies to meet sustainability goals, and in many cases adopting the guidelines provided in the United Nations (UN) Sustainable Development Goals (SDG).

- **Life Below Water** goal is directly linked to the HSC populations and the BET. Aim is to reduce pressure on Marine Biodiversity.

- **Life On Land**: Interconnection is key to also protect Life On Land.

https://sdgs.un.org/goals
TAKEAWAY: REPLACING LAL WITH RECOMBINANT REAGENTS...

• **LAL has been a life-saver** for several decades and HSC protections have been facilitated by LAL manufacturers, but now…

• **Life on Land** > shorebirds are endangered by lack of HSC eggs they need to feed on during migratory journey.

• **Life below water** > HSC is a keystone species with rippling effects if HSCs are further threatened.

• One easy tool to help **alleviate the pressure on the HSC population** = rFC
RECOMBINANT REVOLUTION IS A MOVE FROM…

- Scarcity -> Abundance
- Animal sourcing -> Bioreactor grown (synthetic)
- Empirical /approximate -> Exact science / specific = Modernization
- Local (LAL. Atlantic seaboard) -> Global production
- Farm / shore -> Lab
- Fixed production -> Fully scalable

- Unsustainable -> Sustainable production
WHAT SPECIFICALLY CAN WE DO?

- Move to animal-free products
- Reduce reagent waste (micro & chemistry)
- Reduce plastic waste (or at least do not increase)
- Recycle plastic if possible - difficult when drugs are added to it
WASTE REDUCTION = COST REDUCTION

- We can look at non-animal derived test reagents
  - rFC production follows the paradigm of biologic drug production
  - Waste less by using exactly what you need

Excess LAL can be frozen, but some have seen performance degradation.

The image demonstrates how 4 columns of sample or 32 total wells required approximately 3.2 mL. The lowest amount of LAL that met this volume would require reconstitution of a 5.2 mL vial (or two 2.6 mL vials) and therefore would have wasted 2 mL of LAL for the test. This is a significant amount when considered on a cumulative, annual basis.
LIMIT PLASTIC WASTE

- Single sample test cartridges are 4-5 times more plastic than a 96-well plate (by weight)
- 20-21 cartridges needed to = one 96 well plate
ENDOZYME® II GO – GOPLATE™ OR STRIPS

We can save analyst time and money

- Pre-spiked PPCs, Just add samples
- Add water to reconstitute standard curve
  - No CSE reconstitution
  - It’s in the wells
  - No dilution stds
  - No vortexing stds
  - Just add water
  - Just add samples
CURRENT BIG PHARMA ACTIVITIES

3 CASE STUDIES
**CURRENT BIG PHARMA ACTIVITIES**

**CASE 1**

**A Strategic Approach to Optimisations of Testing Bacterial Endotoxins – by AstraZeneca**

Authors: Dennis Wong, Karen Capper, Miriam Guest, Phil Duncanson – AstraZeneca

AstraZeneca's focus on sustainability is fundamental to its company strategy - part of the mission to meet UN SDG goals.

**Stated Goals**
Strategy for testing endotoxin solutions:
- Reduce the burden on the horseshoe crab
- Reduce waste
- Increase efficiencies
- Minimal change burden

<table>
<thead>
<tr>
<th>Short-term strategy</th>
<th>Long-term strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employed cartridge testing to reduce LAL use</td>
<td>is to employ recombinant reagent testing to eliminate LAL use</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Short-term benefits</th>
<th>Long-term benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume of LAL used reduced by &gt; 60%</td>
<td>validate non-animal derived synthetic alternative reagents</td>
</tr>
</tbody>
</table>
Eli Lilly - Development of rFC as alternative method for endotoxin testing that helps protect wildlife and coastal, marine and watershed environments

Eli Lilly allocated €9M to its ecologically sound endotoxin testing method initiative

Terrestrial and Aquatic Biodiversity Conservation

Expenditures that improve protection of coastal, marine and watershed environments. Example projects may include, but are not limited to:

Development of an alternative method for endotoxin testing that helps protect wildlife and coastal, marine and watershed environments

Life below water

Life on land

The terrestrial and aquatic biodiversity conservation goal for Eli Lilly & Company.

Sustainability Bond Allocation and Impact Report

Terrestrial and Aquatic Biodiversity Conservation

Lilly worked to validate an alternative method of endotoxin testing—a necessary step for our medicines—that does not require sourcing horseshoe crabs. Several species of crabs are under threat or endangered from habitat loss and overharvesting, which negatively affects the ecosystems and other species inhabited by horseshoe crabs. This industry-leading effort helped influence changes in the European pharmacopoeia in 2020 and may help facilitate broader industry adoption and conversion to endotoxin testing alternatives that do not require the use of horseshoe crabs or other live animals.

Impact Metrics:

- Five Lilly medicines were developed using the alternative endotoxin testing method, including three COVID-19 antibodies, and were authorized for use

Proceeds Allocated:

- Ecologically Sound Endotoxin Testing Method
  - €9 million
Validation Strategy for New Recombinant Factor C Users
Authors: Evelyn Der, Carmen Marin, Vivian Grunert de Fonseca

Importance of a Sustainable Alternative Method at Roche

Stated Goals
- **3Rs Incentive Awareness** – awareness for animal welfare: refine, reduce & replace
  - Supports Roche’s Mission: Replace non-sustainable animal dependent analytical methods with alternative methods
- **39,000** water samples annually for endotoxins
  - Alternative methods => significant impact on ANIMAL PROTECTION
USP PHARMACOPEIAL DISCUSSION GROUP (PDG)

USP project to evaluate rFC as Sustainable Solution

Encourage smaller volumes:
- Utilizing and/or reusing lower toxicity solvents
- Revisions to Chapter <31> and <41> (volumetric & balances measurements)

Other ongoing projects

- Evaluation of endotoxin recombinant factor C (rFC) for absence of pyrogen assurance
- Phasing out calomel electrodes (mercury [II] chloride)
- Replacing outdated wet chemistry methods using hazardous reagents
- Eliminating odor/taste aka “organoleptic” tests
- Continuing elimination of toxic solvents
- Analytical quality by design (AQBd) concepts to include reduction in waste generation
Question & Answer