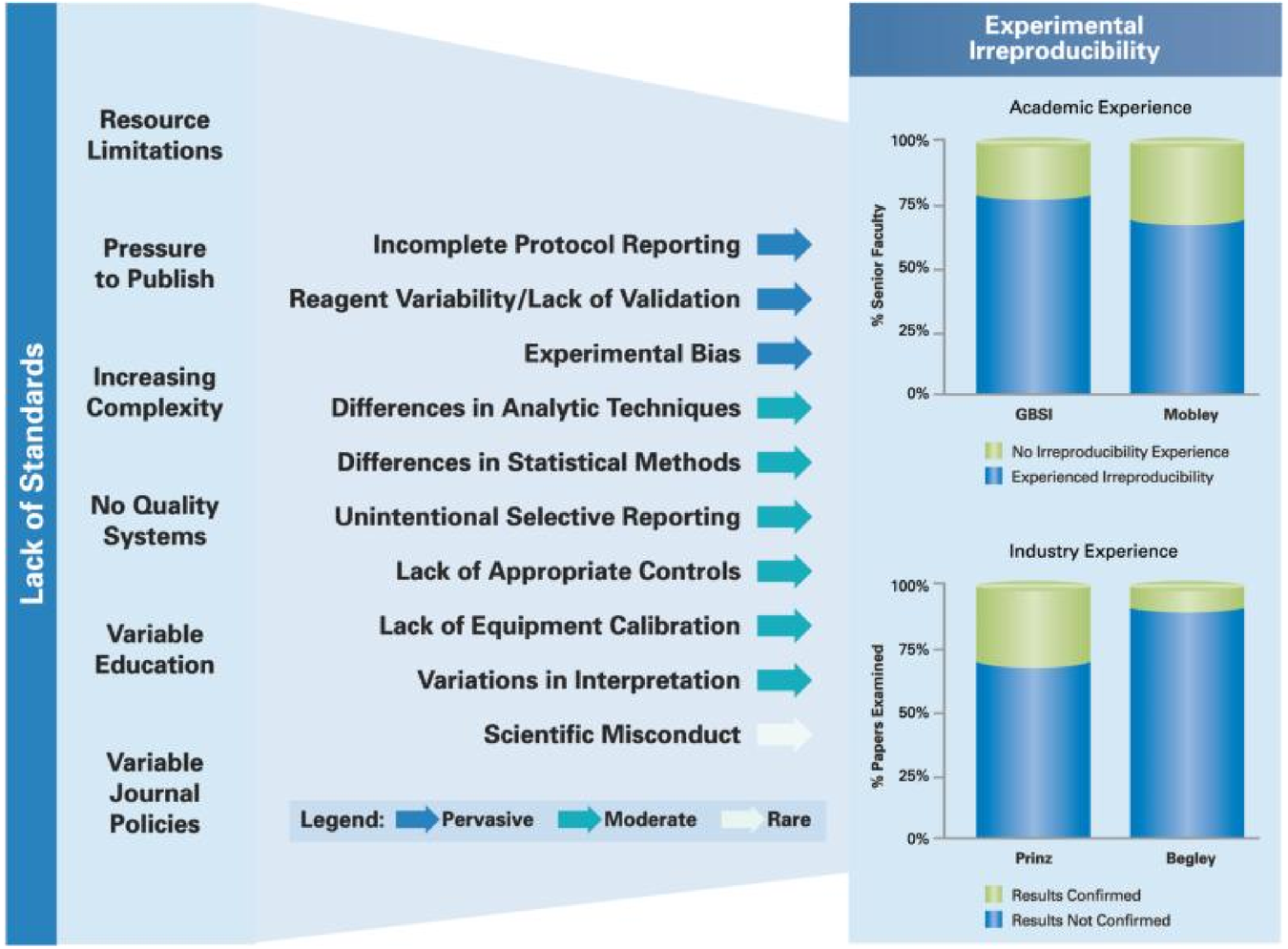
THE CHANGING LANDSCAPE:
PERSPECTIVE ON DATA IRREPRODUCIBILITY OF LIFE SCIENCE RESEARCH TOOLS.

April 18, 2018

Carl A. Ascoli, Ph.D.

Primary Causes of Irreproducibility[1,2,3]

- Study Design
- Biological Reagents
  - Cell lines
  - Antibodies:
    - <50% specific[4,5]
    - $350M loss annually
  - Animal models
  - Other
- Laboratory Protocols
- Data Analysis
- Reporting



[1]Freedman, L.P. et al., *PLOS Biology* **13**(6):1-9 (2015)
[2]Aarssen, L., (2015) http://www.musingsone.com/2015/09/why-most-published-data-are-not.html
[3]Freedman, L.P and Inglese, J., *Cancer Res.* **74**(15):4024-*4029 (2014)
[4]Baker, M., *Nature* **521**:274-276 (2015)
[5]Bradbury, A. and Plückthun, A., *Nature* **518**:27-28 (2015)

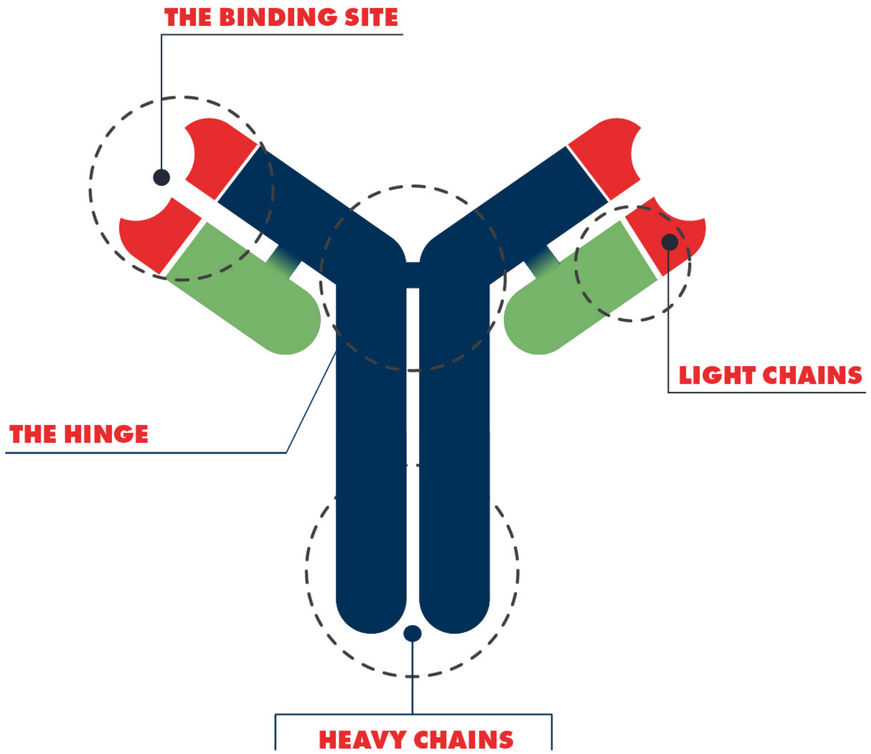
Changes over Time

The Changing Antibody Landscape

- How antibodies are produced; *globally*.
- How researchers think about and use antibodies.
- How antibody-based data is reported.

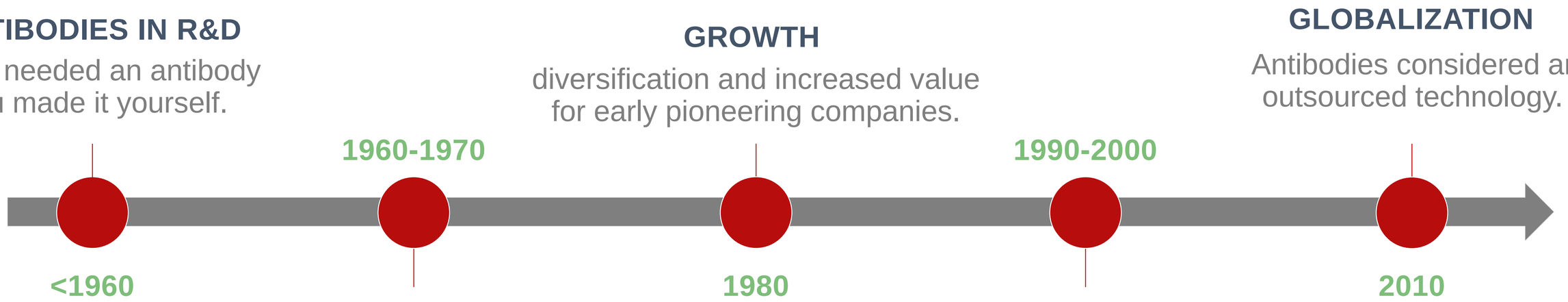
What are Antibodies?

- Specialized proteins of the **immune system** in blood.
- Part of the body's response to **fight pathogens**.
- **Tool of biotechnology;** high sensitivity and specificity.
- Today aid **discovery**, **diagnosis** and **therapy** of diseases.



ROCKLAND™ antibodies & assays

Manufacturers: The Changing Antibody Landscape



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Manufacturers: The Changing Antibody Landscape

GLOBALIZATION

Antibodies considered an outsourced technology

>2010

- ***Supplier extremes*** – literally from Fortune 100 companies to garages in Zhanjiang to hillsides in Katmandu – and everything in between.
- ***OEM Suppliers*** – affects distribution. Producers, distributors, re-sellers, aggregators. Who makes the antibody? Transparency.
- ***Counterfeit Reagents*** – adds additional pressure on supply chain[1]

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[1]Cyranoski, D. *Nature* **545**:148-150 (2017).

The Change in Researcher's Perspectives

The approach of many antibody researchers has changed over time.

- Publish or perish. Time pressure. Many opt to omit **validation steps** before collecting data.[1]
- Many opt to omit **positive and negative controls** from experimental design.
- A **knowledge gap** exists as antibody technology is often outsourced. Result: a disconnect between how antibodies are produced, screened, validated and used.[2]
- Lost concept of "**fit-for-purpose**" antibodies. Not all antibodies will work in all immunoassays.

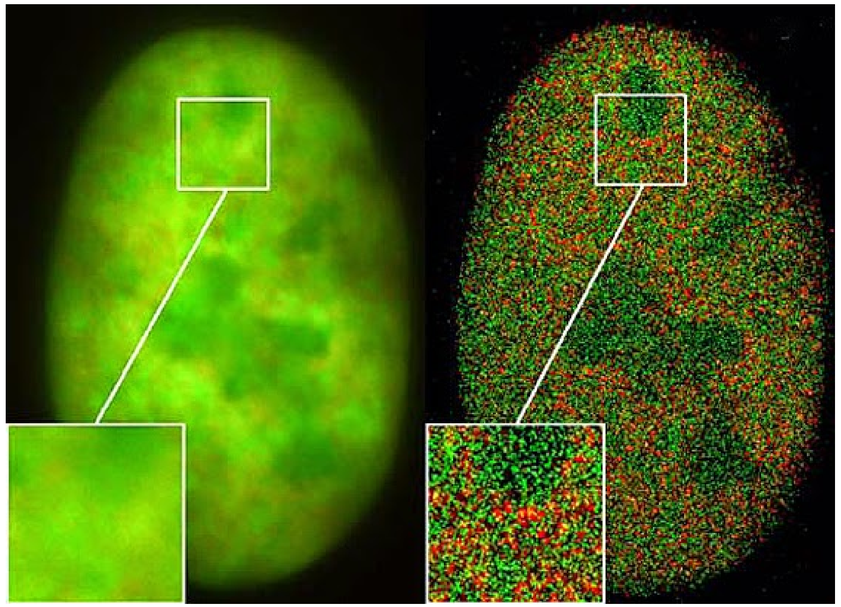
ROCKLAND™ antibodies & assays

[1]Freedman, L.P. et al., *Biotechniques* **61**:16-18 (2016)
[6]Ascoli, C.A. and Birabaharan, J., *Genet. Eng. Biotechnol.* **35(14)**:21-27 (2016

The Change in Immunoassays

Increased demands on how antibodies are deployed.

- **Immunoassay:** an antibody based test that measures or detects a substance, *e.g. pregnancy, cancer, drug abuse*.
- Classic immunoassays like IHC[1] and WB[2] are nearly a **half century old.**
- Today's techniques push the limit of antibody based detection, *e.g. to detect either a single molecule or cell*.
- Require antibodies to be well validated: **sensitivity, specificity and reproducibility** must be defined for each lot of antibody produced.[3]



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[1]Coons AH, et al. *Proc Soc Exp Biol.* **47**:200–2 (1941)
[2]Renart J, et al. *PNAS* **76:**3116-3120 (1979)
[3]Marx, V., *Nature Methods* **10**(8):703-706 (2013)

The Change in Data Reporting

The emphasis of journals changed over time.

- Journals started to move the ‘Methods’ section to the end of a research article, then to a “supplemental” section, and then to “online only”.

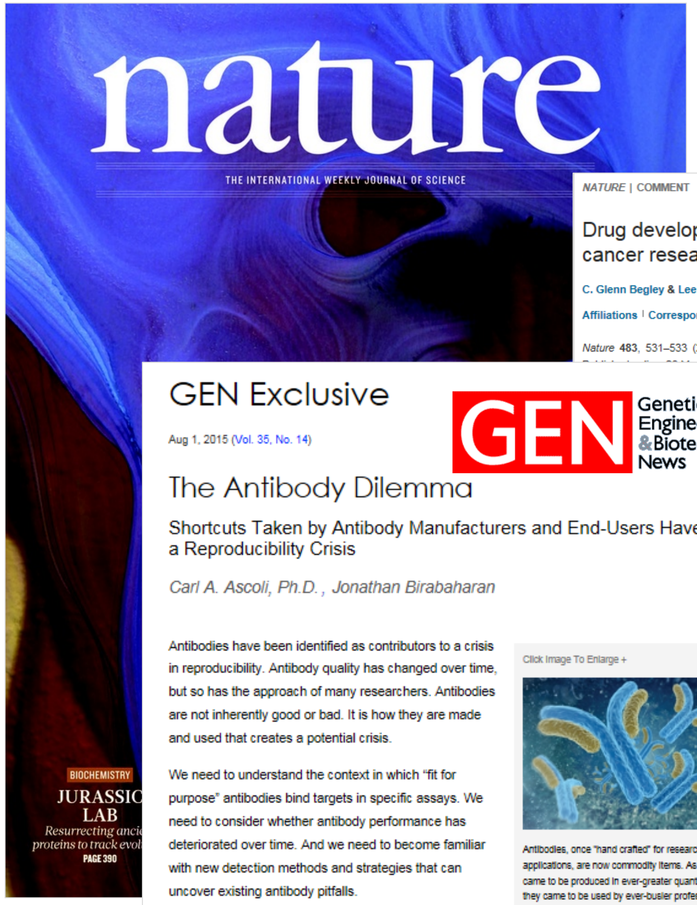
- Proper reporting of reagents’ details and protocols is **critical for reproducibility**. Many reports do not include basic details of critical reagents:

  - *vendor, product description, host species, clonality, part number and lot number.*[2]

- No study is reproducible unless all the technical information is provided in a published article. **The unambiguous identification of antibodies is of utmost importance**.[1]

[1]Weller, M.G., *Anal. Chem. Insights* **11**:21-27 (2016)
[2]Helsby, M.A. et al., *F1000 Research* **2**:153 (2013)

Antibodies in a Firestorm of Controversy



NATURE | COMMENT

Drug development: Raise standards for preclinical cancer research

C. Glenn Begley & Lee M. Ellis

Affiliations | Corresponding author

Nature **483**, 531–533 (29 March 2012) | doi:10.1038/483531a

Should all research antibodies be recombinant monoclonals? Too early to ditch polyclonals!

February 4, 2015 6:00 pm

An article published today in Nature calls for the large scale production of recombinant monoclonal antibodies, along with other recombinant affinity reagents including aptamers and affimers, for use in the research market.

Andrew Bradbury and Andreas Pluckthun argue that this would lead to increased reproducibility and promote progress in biomedical research.

Here at CiteAb we support this initiative, and our founder Dr Chalmers is a co-signatory on the a
we do take issue with the idea that recombinant antibodies should automatically replace polyc

We take a broader view and argue
validation is the key issue – this is
Validation Collection. The best ar

The recombinant monoclonals w
reproducibility. In particular they
sensitive and crucially more spec

NATURE | NEWS FEATURE

Reproducibility crisis: Blame it on the antibodies

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

Monya Baker

19 May 2015

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Illustration by Nik Spencer/Nature

In 2006, things were looking pretty good for David Rimm, a pathologist at Yale University in New Haven, Connecticut. He had developed a test to guide effective treatment of the skin cancer
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d a pattern
relapse after
he clinic.

GEN Exclusive

Aug 1, 2015 (Vol. 35, No. 14)

GEN Genetic Engineering & Biotechnology News

More »

The Antibody Dilemma

Shortcuts Taken by Antibody Manufacturers and End-Users Have Led to a Reproducibility Crisis

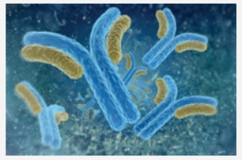
Carl A. Ascoli, Ph.D. , Jonathan Birabaharan

Antibodies have been identified as contributors to a crisis in reproducibility. Antibody quality has changed over time, but so has the approach of many researchers. Antibodies are not inherently good or bad. It is how they are made and used that creates a potential crisis.

We need to understand the context in which "fit for purpose" antibodies bind targets in specific assays. We need to consider whether antibody performance has deteriorated over time. And we need to become familiar with new detection methods and strategies that can uncover existing antibody pitfalls.

Immunoassays such as immunohistochemistry[1] and Western blotting[2] were reported over a half century ago. Today's advanced techniques—ELISA/qPCR, ChIP sequencing, nanoscopy, and nanoimmunoassays—push the limits of antibody performance.

Click Image To Enlarge +



Antibodies, once "hand crafted" for research applications, are now commodity items. As antibodies came to be produced in ever-greater quantity, and as they came to be used by ever-busier professionals, reproducibility became an issue. To improve quality, demanding recombinant approaches have been proposed, but simply paying more attention to monoclonal and polyclonal basics may suffice. [iStock/Svisio]

NATURE | COMMENT

Reproducibility: Standardize antibodies used in research

Andrew Bradbury & Andreas Plückthun

04 February 2015

To save millions of dollars and dramatically improve reproducibility, protein-binding reagents must be defined by their sequences and produced as recombinant proteins, say Andrew Bradbury, Andreas Plückthun and 110 co-signatories.

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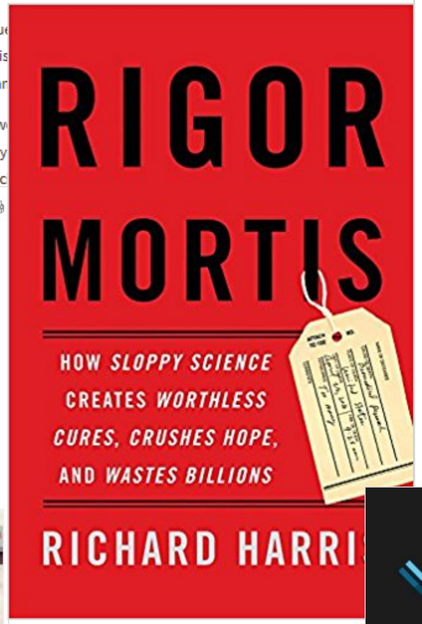
Subject terms: Biotechnology · Cell biology · Databases · Research management



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Journal of Cell Science

A.Bradbury & A. Plückthun. *Nature* **518**, 27–29 (2015).
C.Glenn Begley & Lee M Ellis. *Nature* **483**,531-533 (2012)
CiteAb Blog: http://blog.citeab.com/recombinant-monoclonals-polyclonals/ (Feb 4 2015)
M. Baker. *Nature* **521**:274–276 (2015).
Ying-Li Wu, et al. *Journal of Cell Science* **119**: 2797-2806 (2006).
Ascoli and Birabaharan, Genetic Engineering News **35**; (Aug 1 2015)

The Antibody Dilemma: Data Reproducibility

Problem

- Serious flaws in the reliability of antibodies[8].
- <50% of commercial antibodies recognize ***only*** their target (2008)[7].
- Only 6 of 53 landmark preclinical studies were reproducible (2012)[3].
- Estimated waste in materials, time and money $350M million annually[5].

Recommendations

- Use “recombinant antibodies” only rather than conventional antibodies[4].
- Differentiate research vs clinical use.
- Focus on validation[1,3].
- Focus on fit-for-use deployment[6].
- Create validation standards[2].

[1]Polakiewicz, R.D., *Nature* **518**:483 (2015)
[2]Baker, M., *Nature* **521**:274-276 (2015)
[3]Begley, C.G. and Ellis, L.M., *Nature* **483**:531-533 (2012)
[4]Bradbury, A. and Plückthun, A., *Nature* **518**:27-28 (2015)
[5]Freedman, L.P. et al., *PLOS Biology* **13**(6):1-9 (2015)
[6]Ascoli, C.A. and Birabaharan, J., *Genet. Eng. Biotechnol.* **35**(14):21-27 (2016)
[7]Berglund, L. et al., *Mol. Cell Proteomics* **7**:2019-2027 (2008)
[8]Baker, M., *Nature* **527**:545-549 (2015)

Enough Debate: Time to Act

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The Way Forward: KOL 2015/2016

- **The International Working Group for Antibody Validation** (IWGAV) convened an *ad hoc* committee of international scientists with diverse research interests with the shared goal of improving standards for antibody validation and use.

- **The Global Biological Standards Institute** (GBSI) convened a meeting of over 125 KOL from academia, funding agencies, journals, antibody producers and resellers and related fields to engage all stakeholders[1].

Antibody Validation
Standards, Policies, and Practices

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[1]Uhlen, M. et al., A proposal for validation of antibodies. *Nature Methods* **13;**, 823-827 (2016)

[2]Freedman, L.P. et al., *PLOS Biology* **13(6)**:1-9 (2015)

The Consensus of KOL

- Researchers should include appropriate controls in all immunoassays.
- Researchers should validate critical reagents before collecting experimental data.
- No universally agreed upon immunoassay specific **validation guidelines** exist for antibody production[1]. Creation of guidelines according to “conceptual pillars” is recommended.
- There are **multiple causes** to antibody based data irreproducibility. Antibody producers, researchers, funding agencies, journals and universities have a shared responsibility to **change the *status quo*** about data reproducibility.[2,3]

[1]Bordeuax, J. et al., *Biotechniques* **48(3)**:197-209 (2010)
[2]GBSI Workshop Report: Antibody Validation – Strategies, Policies and Practicves (2016)
[3]Ascoli, C.A. and Birabaharan, J., *Genet. Eng. Biotechnol.* **35(14)**:21-27 (2016)

Changes at Funding Agencies and Journals

- NIH Rigor and Reproducibility guidelines[1,2]:
  - *"Highlight the need to describe details that may have been previously overlooked"*
- Research Councils UK requires data-availability statement for funding.

CellPress

- Cell Press: STAR Methods
  - Structured, Transparent, Accessible Reporting.
  - Required for acceptance of publication.
  - Includes a checklist.

Science
JOURNALS AAAS

nature
International weekly journal of science

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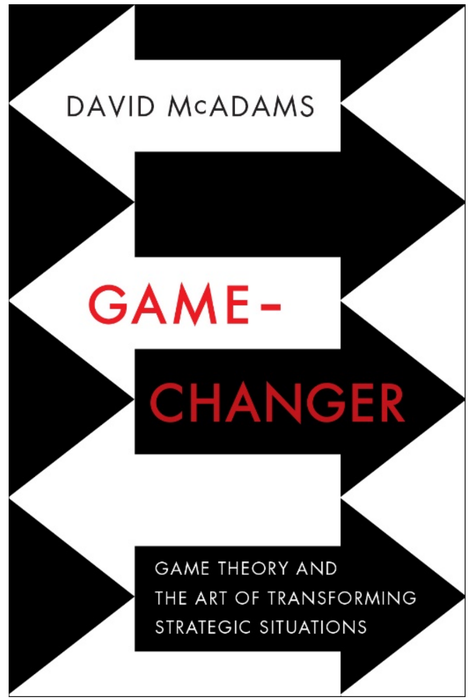
[1]Collins, F.S. and Tabak, L.A., *Nature* **505**:612-613 (2014)
[2] https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research

The Antibody Consortium: 2018

The case for self-regulation of antibody producers and resellers.

- Formative stages of developing a consortium.
- **Game theory**. Early adopter of change.
- Other companies: either follow or embrace *status quo*.
- Market pressure will force change.



Dr. David McAdams,
Fuqua School of Business, Duke University

The Antibody Consortium: 2018

Consortium Mission

- *Advocates for antibody-based science and antibody technologies.*
- *Sets standards for the manufacture and use of antibodies.*
- *Promotes quality and continuous improvement.*
- *Develop mechanisms to assist the sourcing of antibodies.*
- *Assist researchers in being more successful using antibodies.*

Why Self Regulate?

Research Excellence

- Drives significant NIH and NSF funding.
- Innovation results in patented contributions.
- Cannot risk the economic consequences resulting from biotechnology.

LIFE SCIENCES PA

CONNECT · ADVOCATE · ADVANCE

Life Sciences Pennsylvania is a statewide advocate for biotechnology.

Summary

- Antibodies are **one of several identified components** contributing to data irreproducibility.
- Researchers should use **standards and controls** in all experiments and validate critical reagents in their lab first before collecting data.
- Antibody producers and resellers, researchers, funding agencies, journals and universities have a **shared responsibility** to **change the *status quo*.**
- Funding agencies and journals have **started to change** practices.
- Leading antibody producers have committed to setting **standards for validation** and to focus on quality and continuous improvement.

THANK YOU

For more information,
please visit
www.rockland-inc.com
800.656.7625

WHO WE ARE

Rockland. A name synonymous with **quality**, **reliability**, and **service**. Our vision is to advance life science to foster a better world.

www.rockland-inc.com | 800.656.7625

VISION

We advance life science to foster a better world

WHO WE ARE

ESTABLISHMENT

James Fendrick Sr. purchases a company in Rockland County, NY. Relocates to PA. “Rockland Farms” provides animals, animal tissue and blood products.

1962

DEVELOPMENT

1984

Development of secondary antibodies, conjugates and antibody fragments using methods developed by Dr. Cappel at Cappel Laboratoreis and Jackson Immunoresearch.

POLYCLONAL

Production of polyclonal primary antibodies begins.

1990

MONOCLONAL

1995

Building of clean room suites and development of monoclonal antibodies.

NETWORK

Rockland develops multiple paths to the marketplace including OEM and distributorships.

2000

CELL CULTURE

2004

Expansion to cell culture, molecular biology and solutions production.

LIMERICK

Moved production and research to new facility in Limerick, PA.

2014

ANIMAL FACILITY

2017

Expands to state of the art animal facility in Limerick, PA.

ROCKLAND™ antibodies & assays

COMMUNITY

Philadelphia Mural Arts Program

Joy Capel Young Investigator Award

Alex’s Lemonade Stand

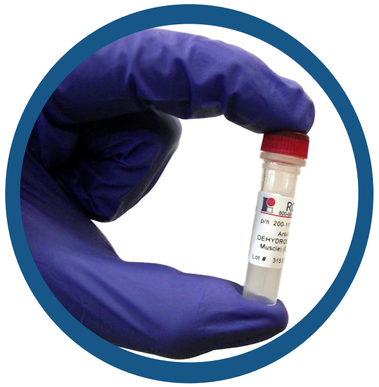
Internship Program

MCCC-Biotechnology program

We provide solutions for

ROCKLAND™ antibodies & assays

WHAT WE DO



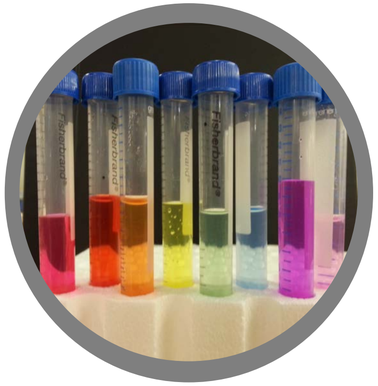
Catalog Reagents
& Bulk Services

Antibody & Protein
Generation

Purification &
Modifications

Cell Culture

Assay Development



Specialized
Services

ROCKLAND™
antibodies & assays

CATALOG PRODUCTS

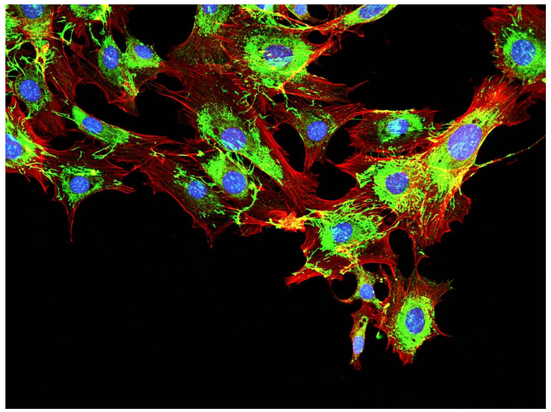
- Primary Antibodies
- Secondary Antibodies
- Buffers & Substrates
- Cell Banking & Cell Supply
- Assay Kits
- Proteins & Peptides
- Blood Products

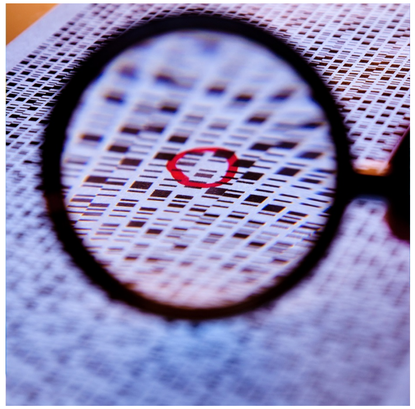
Leading Reagents Supplier with over 10,000 off the shelf products, and custom bulk sizes availalble

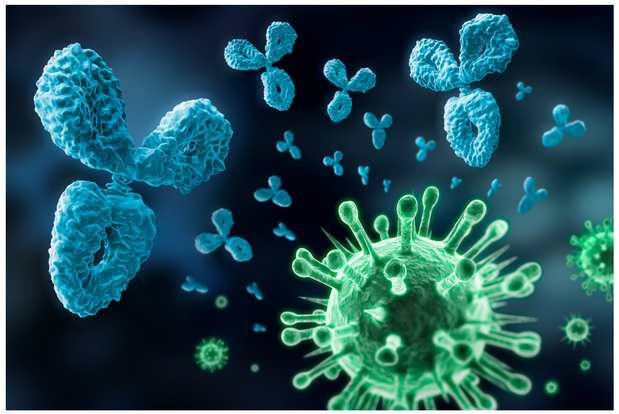
ROCKLAND™ antibodies & assays

CUSTOM SERVICES AND BULK PRODUCTS

- Antibody & Protein Generation
- Purification & Modification
- Molecular Biology
- Cell Culture & IHC
- Assay Development
- Analytical Services
- Other Specialized Services







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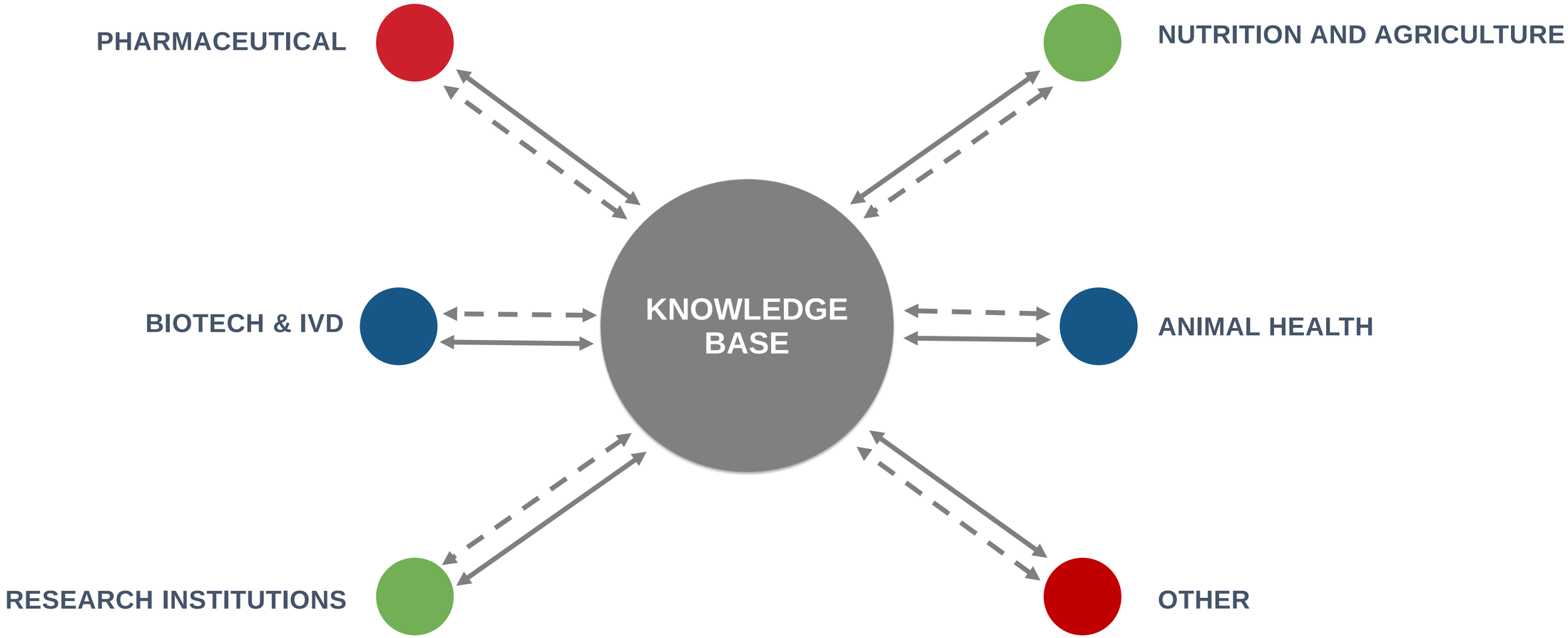
WE ARE A SOLUTIONS PROVIDER, BUT WE SUPPLY MORE THAN JUST REAGENTS



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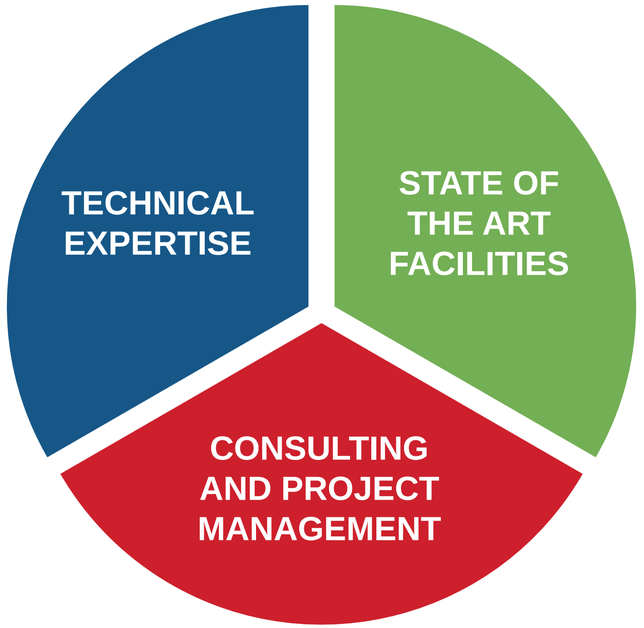
ANTIBODY DEVELOPMENT

Technical Expertise

- Over 3 decades producing Abs
- Complete on site capability from immunization, production, purification, conjugation, fragmentation and characterization

Consulting and Project Management

- Products & Services
- Design
- Kickoff and Closeout
- Partnership style interaction



State of the Art Facilities

- R&D
- Manufacturing
- Animal Facility for:
  - Rodents
  - Rabbits
  - Chickens
  - Goats
  - Sheep
  - Donkeys
  - Llamas
  - Cows
  - Horses

ROCKLAND™ antibodies & assays

SOLUTIONS-DRIVEN PROVIDER

Example of a current project:

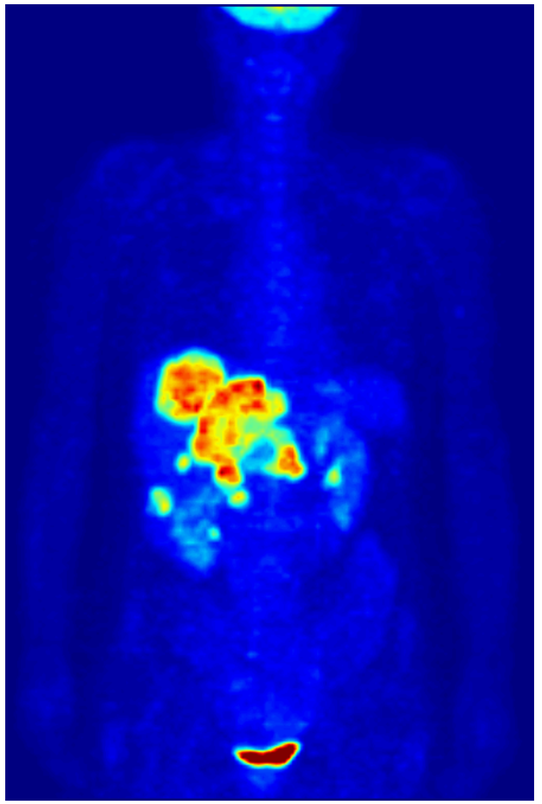
Systemic Pre-targeted Radioimmunotherapy with Bispecific Nanobodies (BsAb) for Cancer Treatment

We partnered for the theranostic project with Dr. Carolyn Anderson at the University of Pittsburg

Theranostics:

A “**thera**py” and “diag**nostics**”

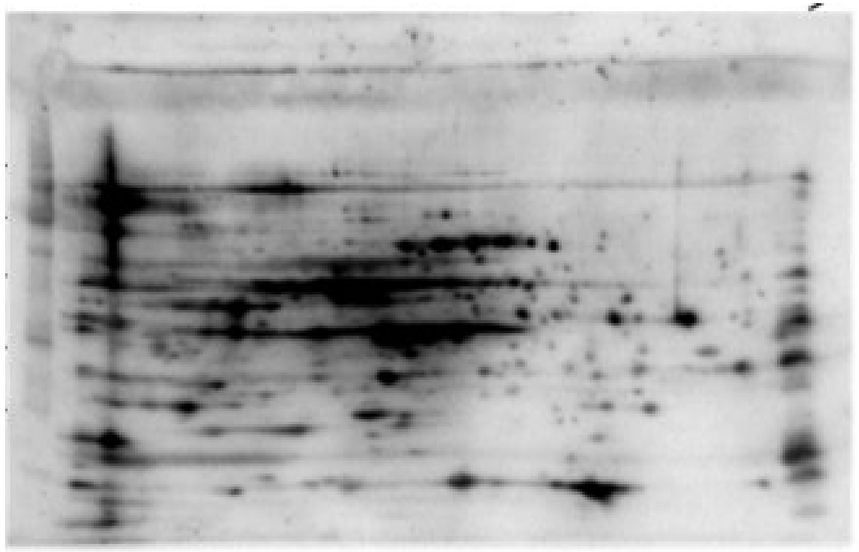
- Detecting Tumors, more effective strategy
- Low toxicity for patient
- Increased sensitivity
- Low background
- Faster uptake and clearance of free radionuclides



https://en.wikipedia.org/wiki/Positron_emission_tomography

SOLUTIONS-DRIVEN PROVIDER: HOST CELL PROTEINS

Case Study: Client had been unsuccessful in generating a specific HCP antibody for an FDA clearance assay for their new therapeutic antibody. They came to Rockland to generate the reagent and assay.



> *Our previous process specific attempt was unsuccessful and we are behind on assay development. Can you help create an approach to fulfill our need for an anti-HCP reagent and assay?*

Rockland successfully solved the reagent generation problem and the work has progressed into development of a process-specific assay

Spark™ THERAPEUTICS

ROCKLAND™ antibodies & assays

SOLUTIONS-DRIVEN PROVIDER

Case Study: A large client was preparing for clinical trials when they discovered that the antibodies required for their companion diagnostic did not exist commercially. They came to Rockland to develop and manufacture antibodies specific to their needs.

> *"The AKT isoform antibodies work great. The AKT3 specific antibody is the only isoform specific antibody that actually works."*

We are your partner for "fit for purpose" projects.

ROCKLAND antibodies & assays

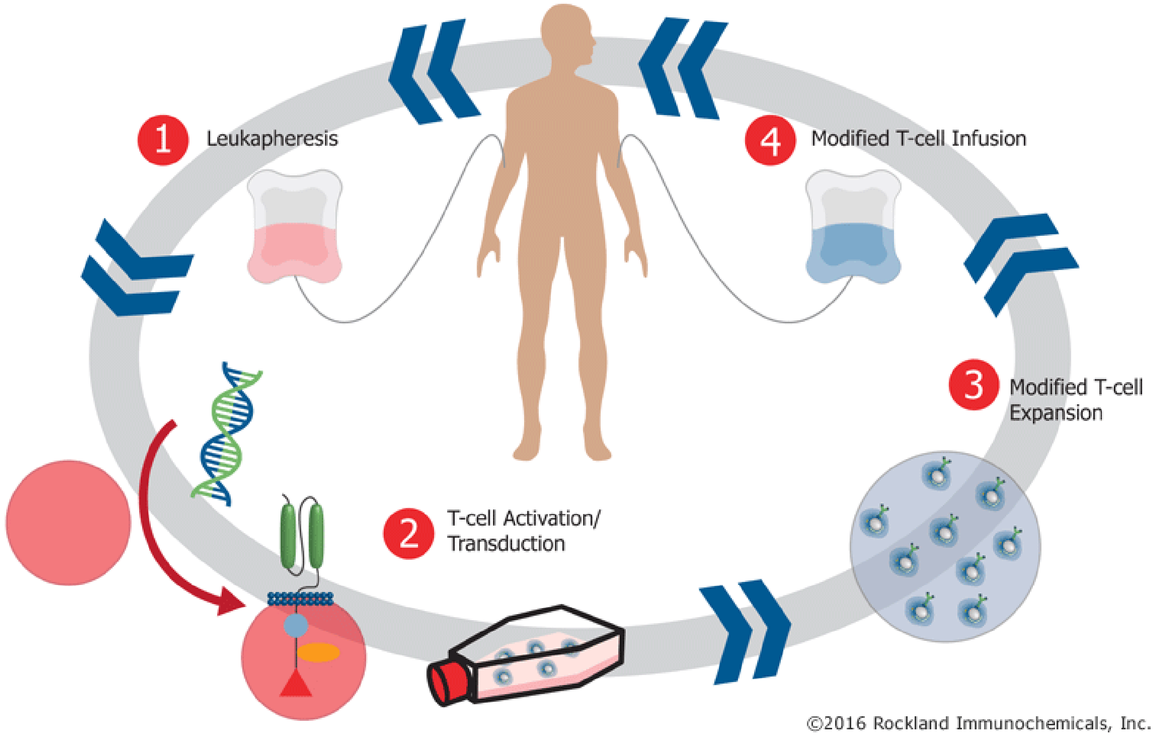
leidos

SOLUTIONS-DRIVEN PROVIDER: IMMUNO-ONCOLOGY

Case Study: Client had licensed CAR-T technology and needed scientists and a laboratory to complete a proof of concept of the technology and a pilot for production.

> *Rockland stepped-up and executed on all the work for us. The technical transfer, sourcing the staff, managing the logistics, and performing the science to our specifications.*

Tech transfer, study design, trouble-shooting, reporting… Rockland can be your principal partner to help you complete the hard projects.



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SOLUTIONS-DRIVEN PROVIDER: INNOVATION CATALYST

Case Study: Start-up company wanted to demonstrate use of cows to produce unique therapeutic antibodies.

> *You did a great job with our proof of concept effort, we'd like to work with you to solve how we can go to large scale efforts…*

Rockland became a consultant and key partner in taking a small scale pilot study into large-scale manufacturing.

ROCKLAND™ antibodies & assays

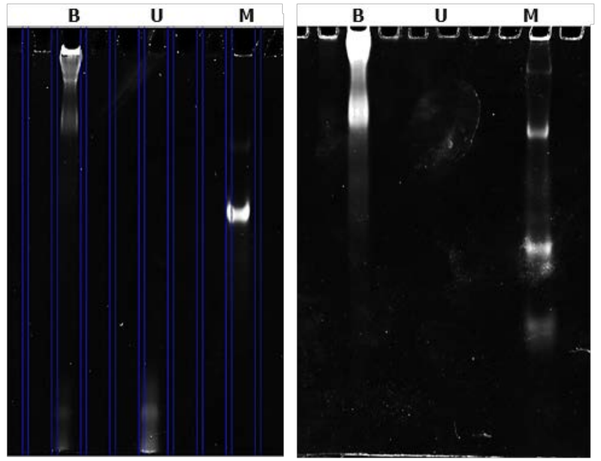
SOLUTIONS DRIVEN PROVIDER: ANTISENSE OLIGO (ASO) ANTIBODIES

ASO antibodies developed by Rockland successfully support characterization studies of antisense drugs

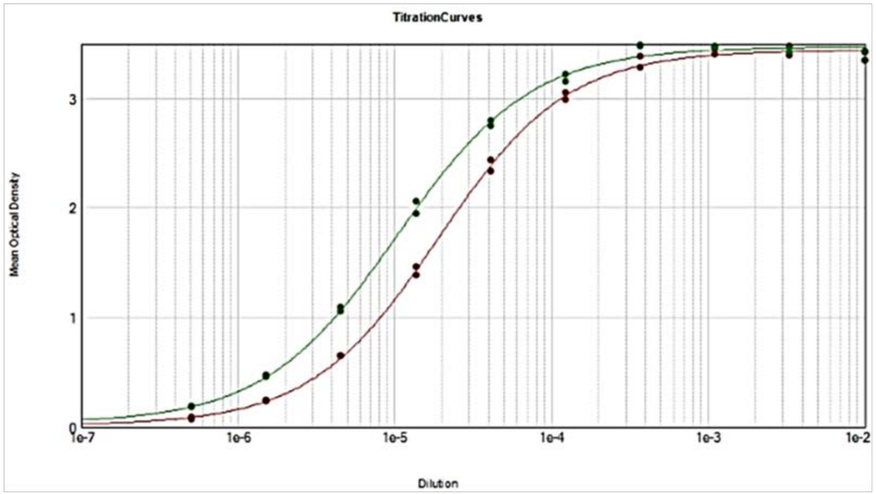
> *We use Rockland for the difficult projects like anti-DNA and anti-oligo projects because Rockland has demonstrated success and understands our timelines.*

Rockland antibody know-how makes us a best in class analytical reagent partner

ASO Conjugation



ASO Antibody



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THANK YOU

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