# Clinical Laboratory Meets Clinical Care:

## Challenges with neural autoantibody test utilization, interpretation, and application in clinical care

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# Learning Objectives

Recognize common pitfalls when neural autoantibody testing is ordered

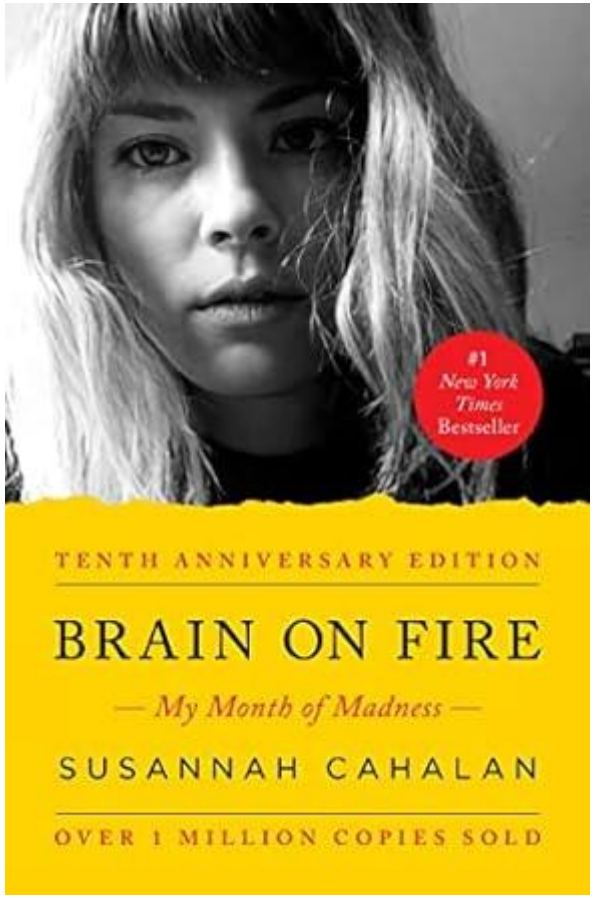
Compare testing methods and explain how these impact the interpretation of laboratory results

Identify opportunities to improve neural autoantibody test utilization in your own laboratory

# Learning Objectives


Credit: Shutterstock

# WHY IS NEURAL AUTOANTIBODY TESTING SUCH A BIG DEAL?




NMDAR antibody encephalitis

ARTICLE | July 18, 2017 |

## IgLON5 antibody

### Neurological accompaniments and outcomes in 20 patients

Josephe A. Honorat, MD, PhD, Lars Komorowski, PhD, Keith A. Josephs, MD, Kai Fechner, MEng, Erik K. St Louis, MD, MS, Shannon R. Hinson, PhD, Sabine Lederer, Neeraj Kumar, MD, Avi Gadoth, MD, Vanda A. Lennon, MD, PhD, Sean J. Pittock, MD, and Andrew McKeon, MD | AUTHORS INFO & AFFILIATIONS

September 2017 issue • 4 (5) • https://doi.org/10.1212/NXI.0000000000000385

## Investigations in $GABA_A$ receptor antibody-associated encephalitis

VIEW EDITORIAL

Marianna Spatola, MD, Mar Petit-Pedrol, BS, Mateus Mistieri Simabukuro, MD, Thaís Armangue, MD, PhD, Fernanda J. Castro, MD, Maria I. Barcelo Artigues, MD, Maria R. Julià Benique, MD, Leslie Benson, MD, Mark Gorman, MD, Ana Felipe, MD, Ruben L. Caparó Oblitas, MD, Myrna R. Rosenfeld, MD, PhD, Francesc Graus, MD, PhD, and Josep Dalmau, MD, PhD SHOW FEWER | AUTHORS INFO & AFFILIATIONS

March 14, 2017 issue • 88 (11) 1012-1020 • https://doi.org/10.1212/WNL.0000000000003713

# NEW NEURAL AUTOANTIBODIES AND ASSOCIATED SYNDROMES ARE REPORTED EACH YEAR

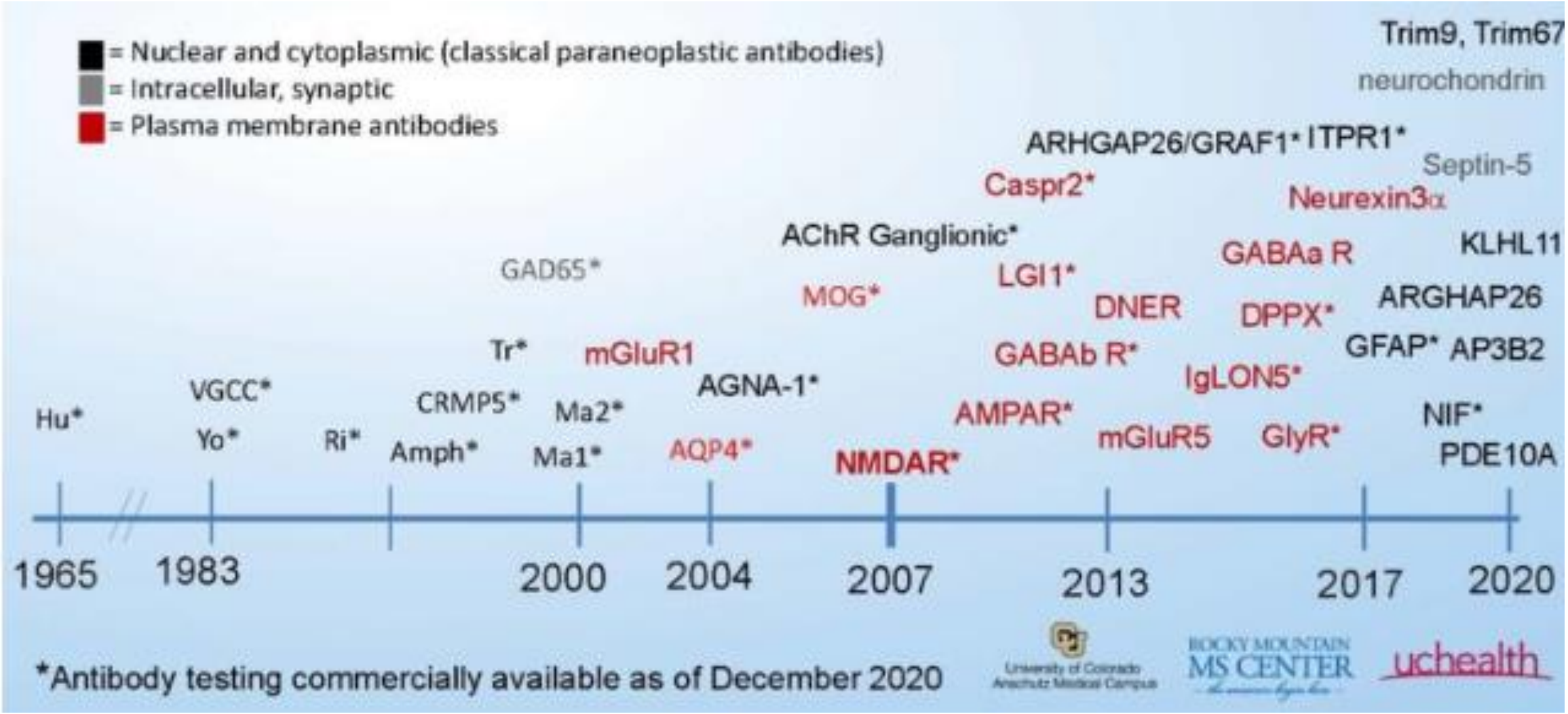



Image from https://aealliance.org/ae-today/

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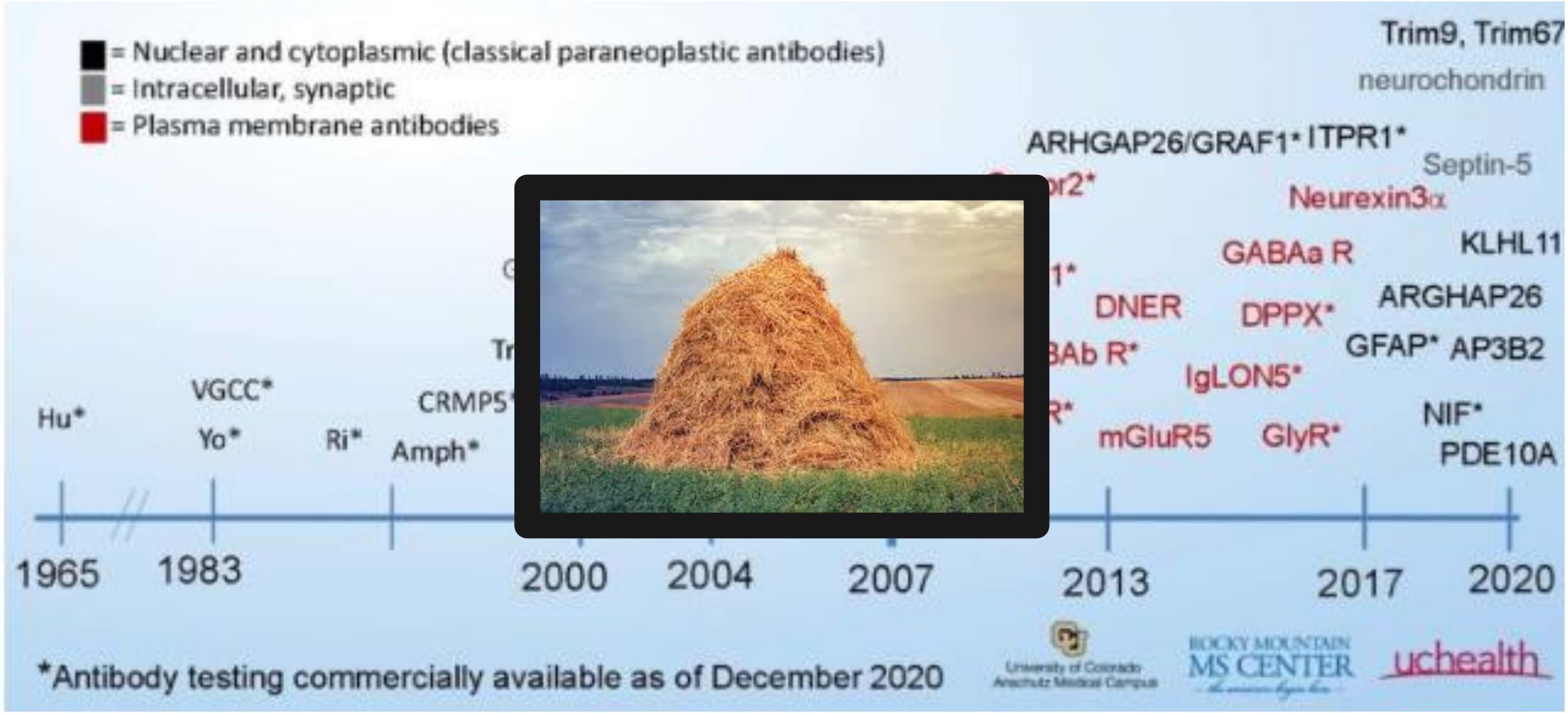


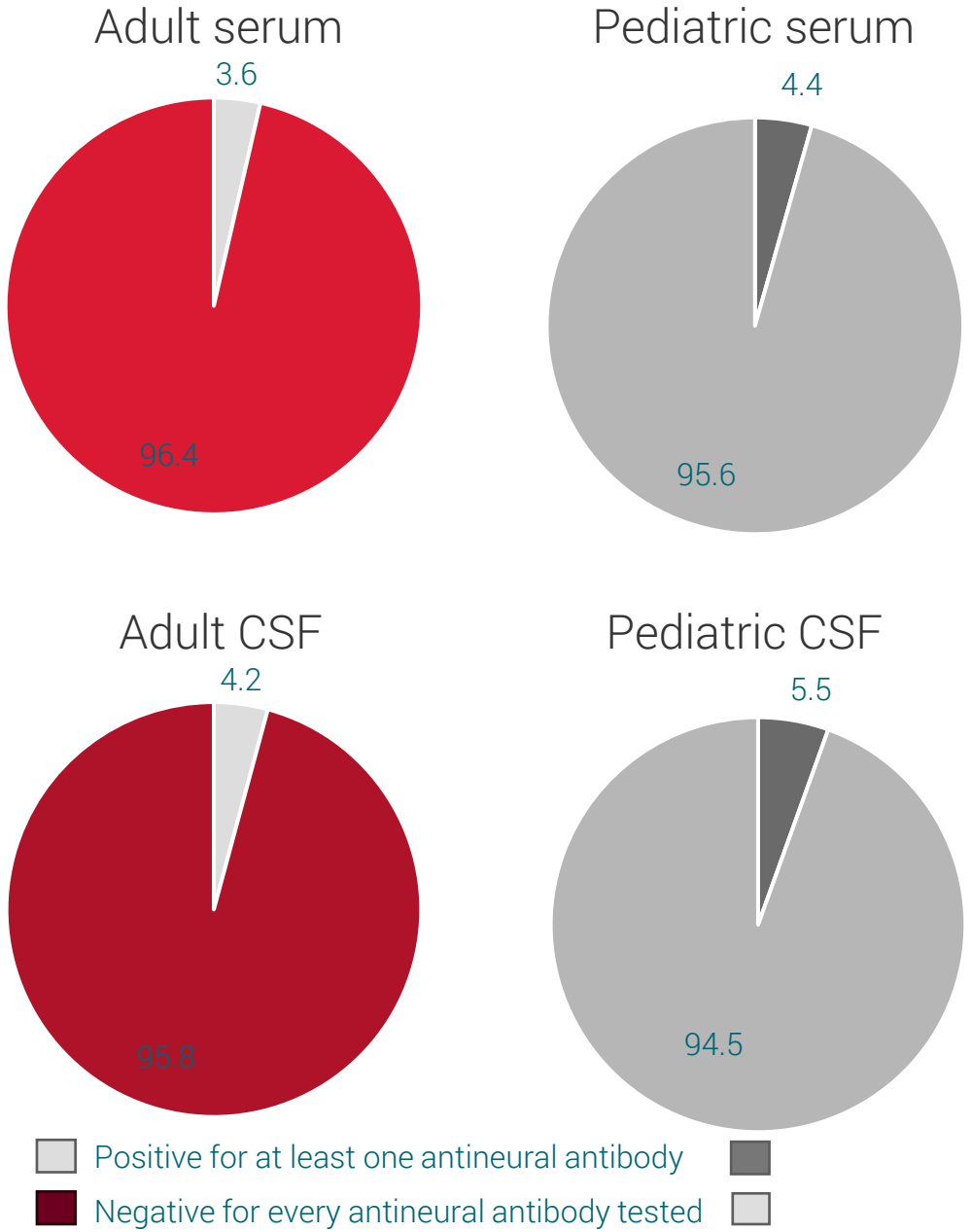

Image from https://aealliance.org/ae-today/

# POSITIVE RESULTS ARE RARE!

Image created using Adobe Firefly

About 95% of all autoimmune encephalopathy panel evaluations are negative for every antibody tested

(Extracted from data in Kunchok et al. Mayo Clin Proc. 2022;97(3):547059)



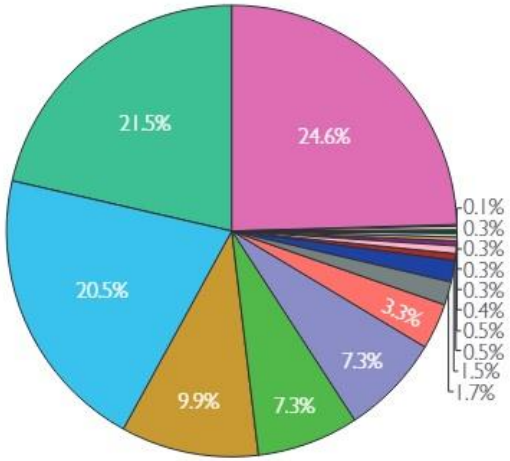
# MOST POSITIVES OCCUR IN A SMALL GROUP OF ANTIBODIES

Image created using Adobe Firefly

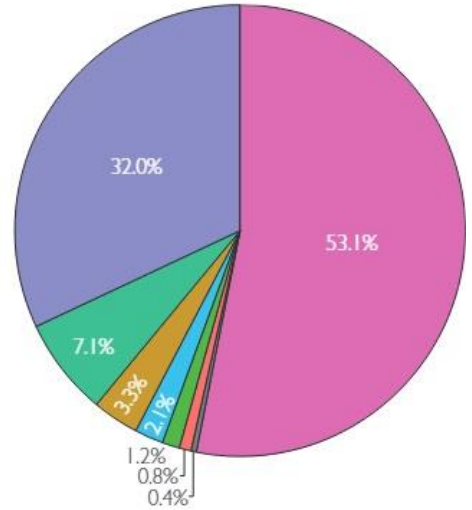
Of the samples positive for any autoantibody, >95% are represented by 8 antibodies

(Kunchok et al. Mayo Clin Proc. 2022;97(3):547059)

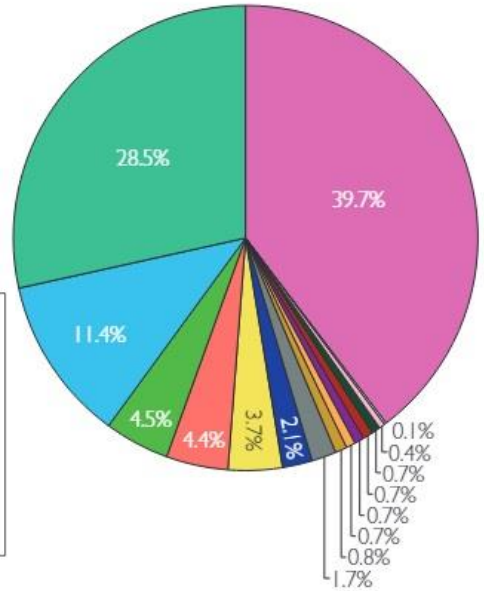
Adult serum



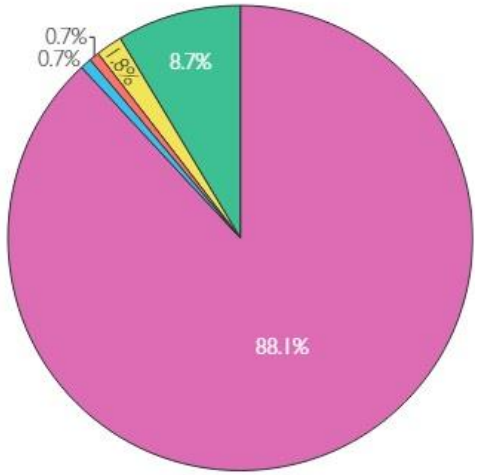

Pediatric serum

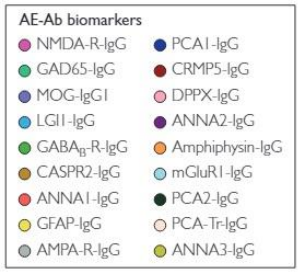



Adult CSF




Pediatric CSF



# Improving clinician ordering patterns is a huge opportunity to improve patient care!


Credit: Shutterstock

# Common Pitfalls When Neural Autoantibody Testing is Ordered

- Broadest available panel is ordered

  "My patient is sick, I want to check all of the things to make sure I'm not missing anything."

- Paraneoplastic Panel is ordered

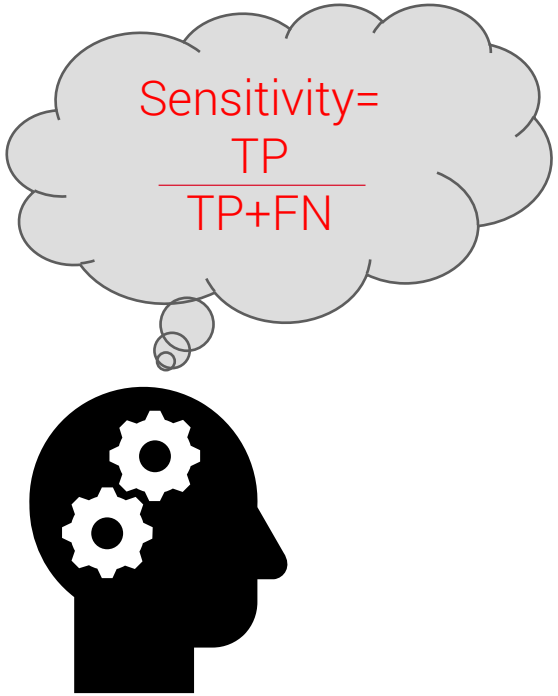
  "Aren't paraneoplastic antibodies and neural autoantibodies the same thing?"

- The wrong phenotype-specific panel is ordered, or multiple redundant panels are ordered

  "There are so many antibody panels, I can't keep them all straight. I picked my favorite panel a long time ago and stick with that."

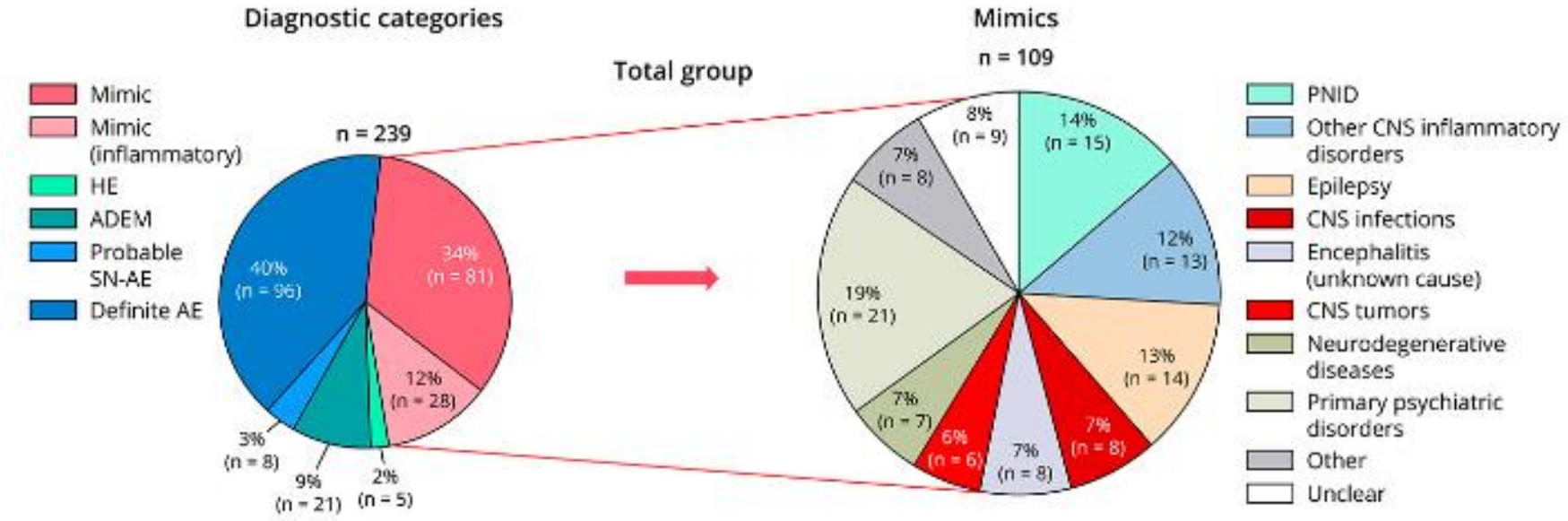
# Pitfalls of "All-Inclusive" Antibody Panels

- Sensitivity, Specificity, PPV, NPV are tricky with neural autoantibody testing



# Pitfalls of "All-Inclusive" Antibody Panels

- Many other diseases may mimic autoimmune encephalitis & false positives are not uncommon, results must be validated by another method (especially in serum!) and considered in the clinical context of the patient




https://doi.org/10.1212/NXI.0000000000200148

# Pitfalls of "All-Inclusive" Antibody Panels

- Sensitivity, Specificity, PPV, NPV are tricky with antineural antibody testing
- Many other diseases may mimic autoimmune encephalitis, false positives are not uncommon (estimated at 10%)
- All-inclusive does not actually have EVERY antineural antibody
  - » Some antibodies are patented, only available at certain labs

# Pitfalls of "All-Inclusive" Antibody Panels

- Sensitivity, Specificity, PPV, NPV are tricky with antineural antibody testing
- Many other diseases may mimic autoimmune encephalitis, false positives are not uncommon (estimated at 10%)
- All-inclusive does not actually have EVERY antineural antibody
- Overinterpreting positive antibody results can expose patients to
  - » Unnecessary immunotherapy
  - » Costly and invasive cancer screening
  - » Delayed accurate diagnosis

# Paraneoplastic Panels

- Vary by clinical reference lab
- “Classic” high-risk for cancer antibodies
  - » Hu, Ri, Yo, Tr/DNER, CRMP5, Amphi, SOX1

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Case: 64yo male with subacute onset of limbic encephalitis.

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- Not phenotype-specific (misses autoantibodies with similar phenotypes but not high-risk for cancer)
- Delays ordering the right test and may give a false sense of security that "antibodies were checked"
- Why are these still ordered? Habit, misunderstanding
- Why are these still available? Because they keep being ordered

# The Wrong Phenotype-Specific Panel is Ordered

- Ordering patterns (“favorites” lists in EMRs and Order Sets) can lead to the same panel being ordered regardless of clinical phenotype.

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Case: 66yo male following up an abnormal GABAa result from OSH

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Case: 66yo male following up an abnormal GABAa result from OSH

| Test Name (Test Number) | Cell Surface and Synaptic Antibodies | | | | | | | | | | | | | | | | | | | | | Intracellular Antibodies | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AChR Binding Ab, IgG | AChR Blocking Ab, IgG | AChR Modulating Ab, IgG | AMPAR Ab, IgG | AQP4 Ab, IgG | CASPR2 Ab, IgG | DPPX Ab, IgG | GABA-AR Ab, IgG | GABA-BR Ab, IgG | Ganglionic AChR Ab, IgG | IgLON5 R Ab, IgG | LGI1 R Ab, IgG | mGluR1 Ab, IgG | MOG1 Ab, IgG | NMDAR Ab, IgG | N-Type VGCC Ab, IgG | P/Q-Type VGCC Ab, IgG | PCCA-Tr/DNER | Striated muscle Abs, IgG | Titin Ab, IgG | VGKC Abs, IgG | Amphiphysin Ab, IgG | ANNA-1 (Hu) | ANNA-2 (Ri) | CV2 (CRMP-5) Ab, IgG | GAD65 Ab | ITPR1 Ab, IgG | PCCA-1 (Yo) | Recoverin Ab, IgG | SOX1 (AGNA) Ab, IgG | TAT (days) |
| Autoimmune Encephalopathy/Dementia Panel, Serum (3006201)[b] | | | | ✓ | | ✓ | ✓ | | ✓ | | ✓ | ✓ | ✓ | | ✓ | | | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | 3–10 |
| Autoimmune Epilepsy Panel, Serum (3006204)[b] | | | | ✓ | | ✓ | ✓ | ✓ | ✓ | | | ✓ | ✓ | | ✓ | | | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | 3–10 |
| Autoimmune Movement Disorder Panel, Serum (3006206)[b] | | | | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | 3–10 |

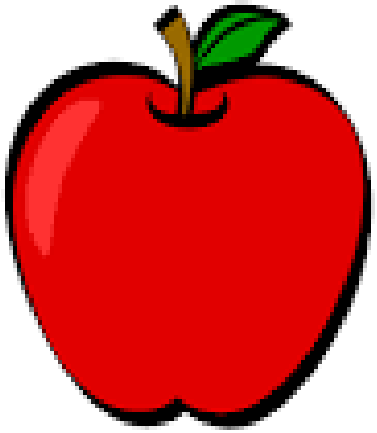
# Opportunities to Improve Clinical Care

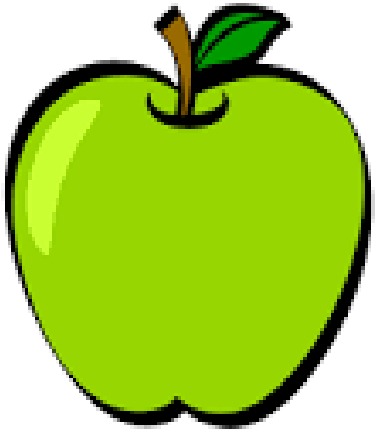
- Establish relationships between clinical laboratory medicine and neurology (or other specialties ordering testing)
- Discuss ordering patterns (bring data!) and educate about major pitfalls (paraneoplastic testing, broad “comprehensive” panels, wrong panel)
- Consider cross-department QI projects

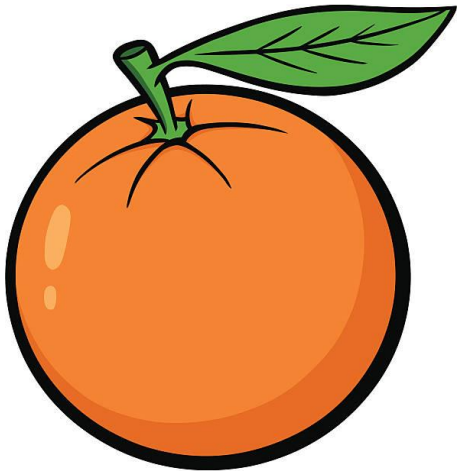
Image from iStock

- Goal: Improve patient care!

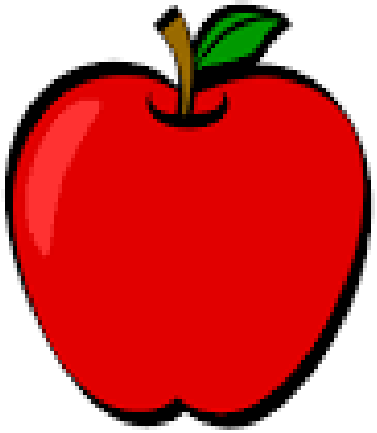
# Testing Methods Vary



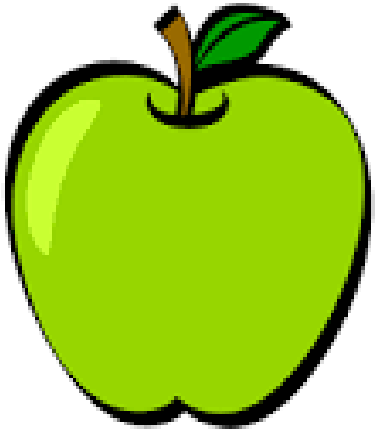




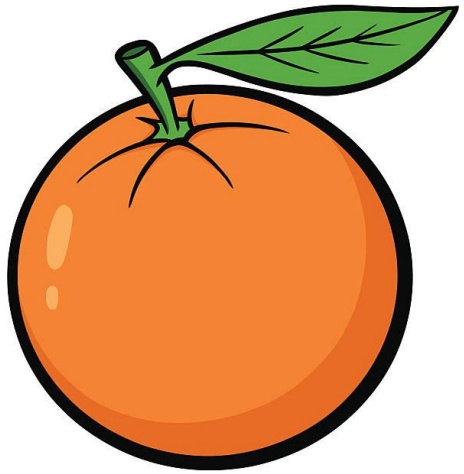
# Testing Methods Vary-AQP4



Fixed CBA



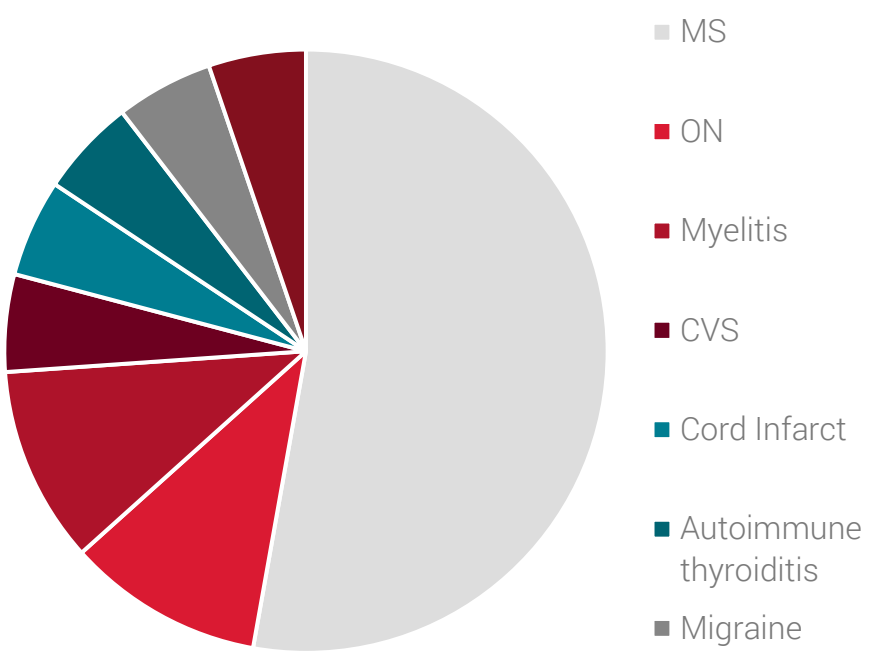
Live CBA



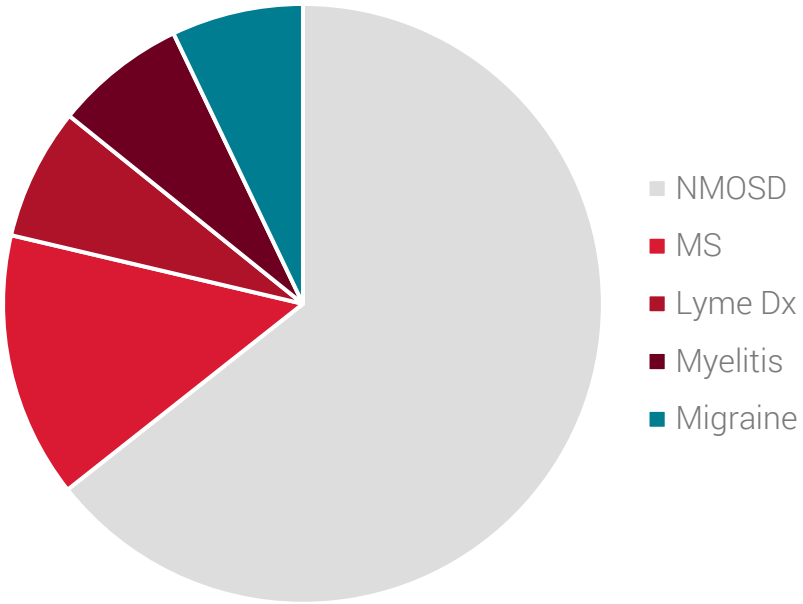
ELISA

# Testing Methods Vary- AQP4 (ELISA)

Low Pos (3-7.9 U/mL)



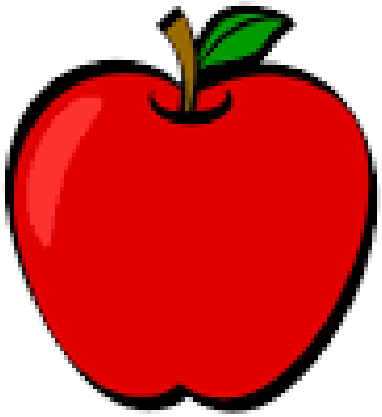

Mod Pos (8.0-79.9 U/mL)



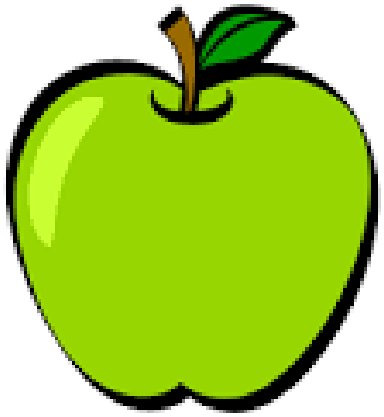

>80 U/mL 100% specific for NMOSD

Adapted from
doi: 10.1155/2021/8692328

# Testing Methods Vary- AQP4



Fixed CBA



Live CBA

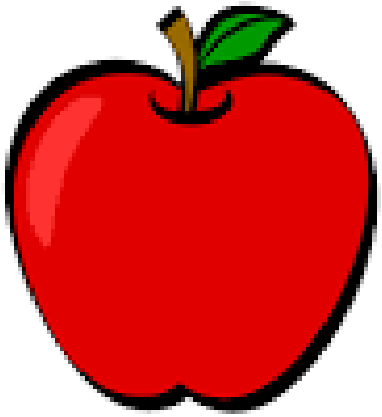
| 2012 | Live FACS | Ox Live CBA | EI fixed CBA |
|---|---|---|---|
| Sens | 77% (46/60) | 73% (44/60) | 68% (41/60) |

https://doi.org/10.1212/WNL.0b013e318248dec1

| 2019 | Ox Live CBA | EI fixed CBA |
|---|---|---|
| Sens | 92% (33/36) | 94% (34/36) |
| Spec | 100% (49/49) | 98% (42/43) |

doi: 10.3389/fneur.2019.01028

# Testing Methods Vary- AQP4



Fixed CBA

Range in an earlier study:
Sensitivity 80.3%-93.9%
Specificity 85.8%-100%

doi:10.1136/jnnp-2015-312601

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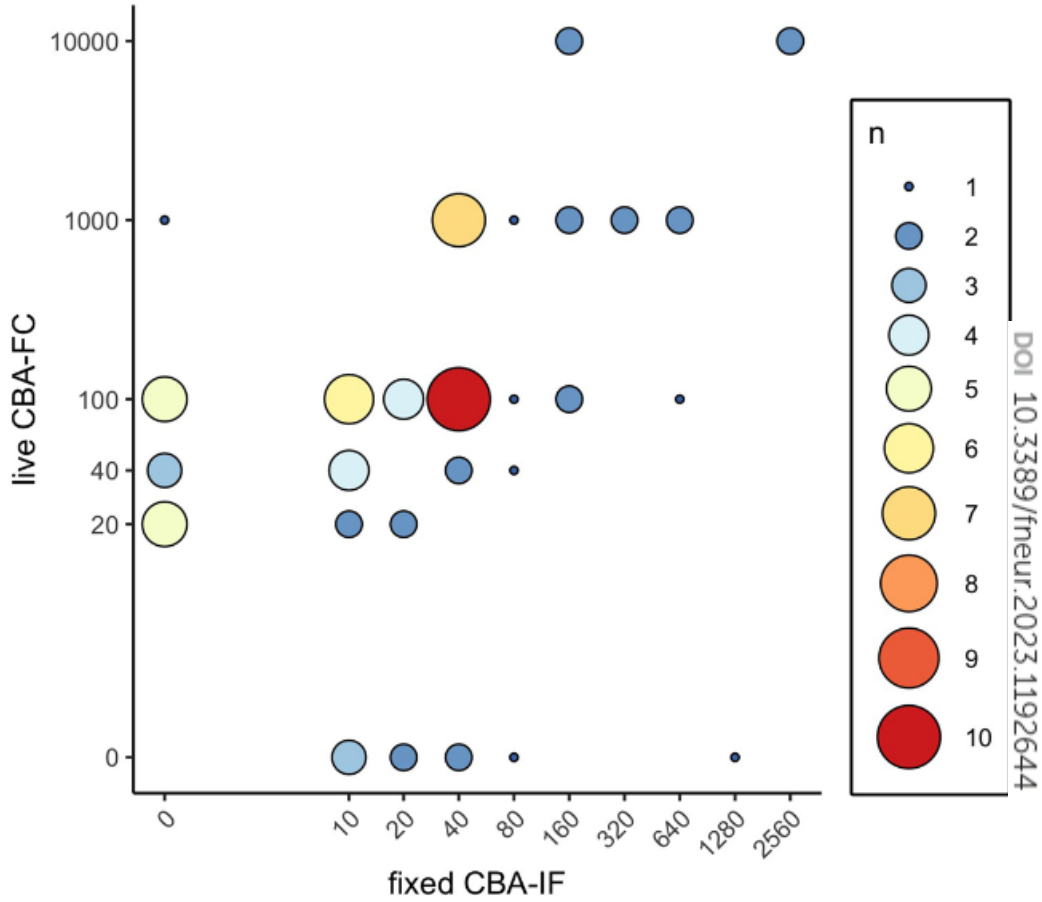
# Testing Methods Vary- MOG

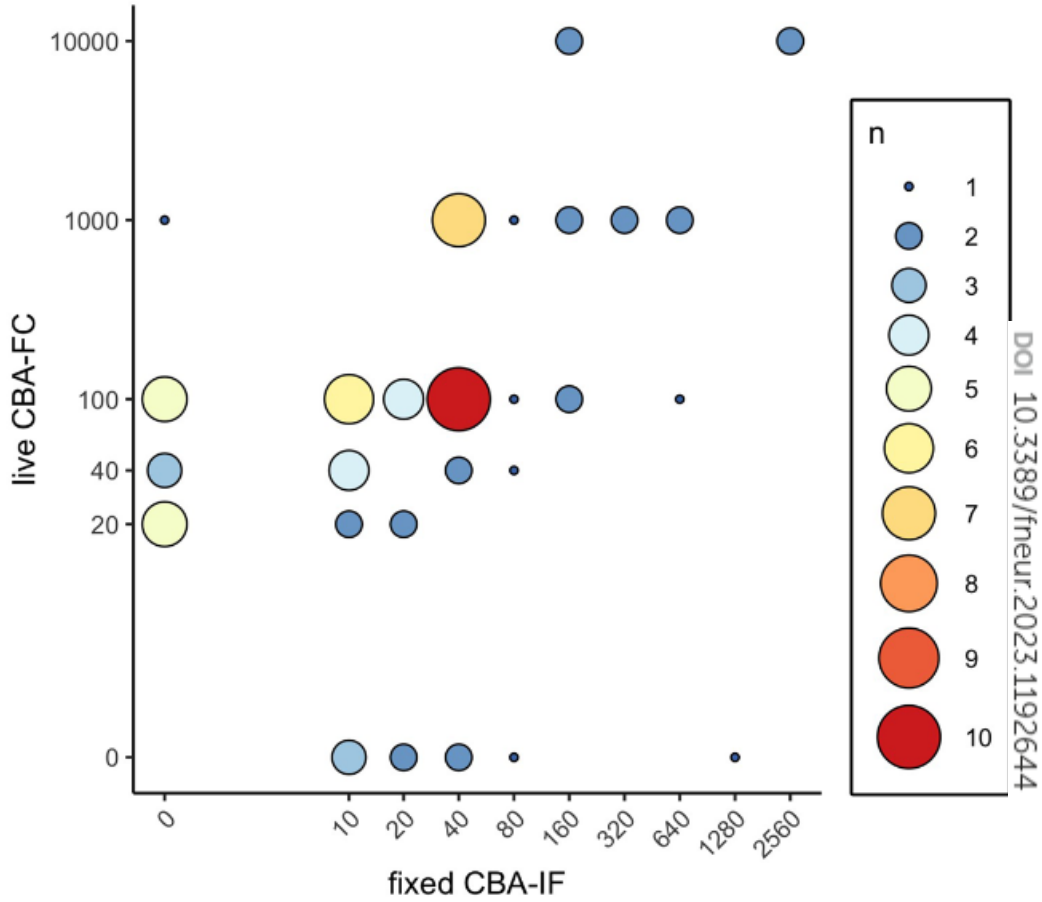
- 93% agreement between paired live and fixed CBA for MOG (no clinical information to determine sens/spec)



# Testing Methods Vary- MOG

- 93% agreement between paired live and fixed CBA for MOG (no clinical information to determine sens/spec)
- Live CBA shown to be
  - » 51% PPV at 1:20-1:40
  - » 82% PPV at 1:100
  - » 100% PPV at ≥1:1000

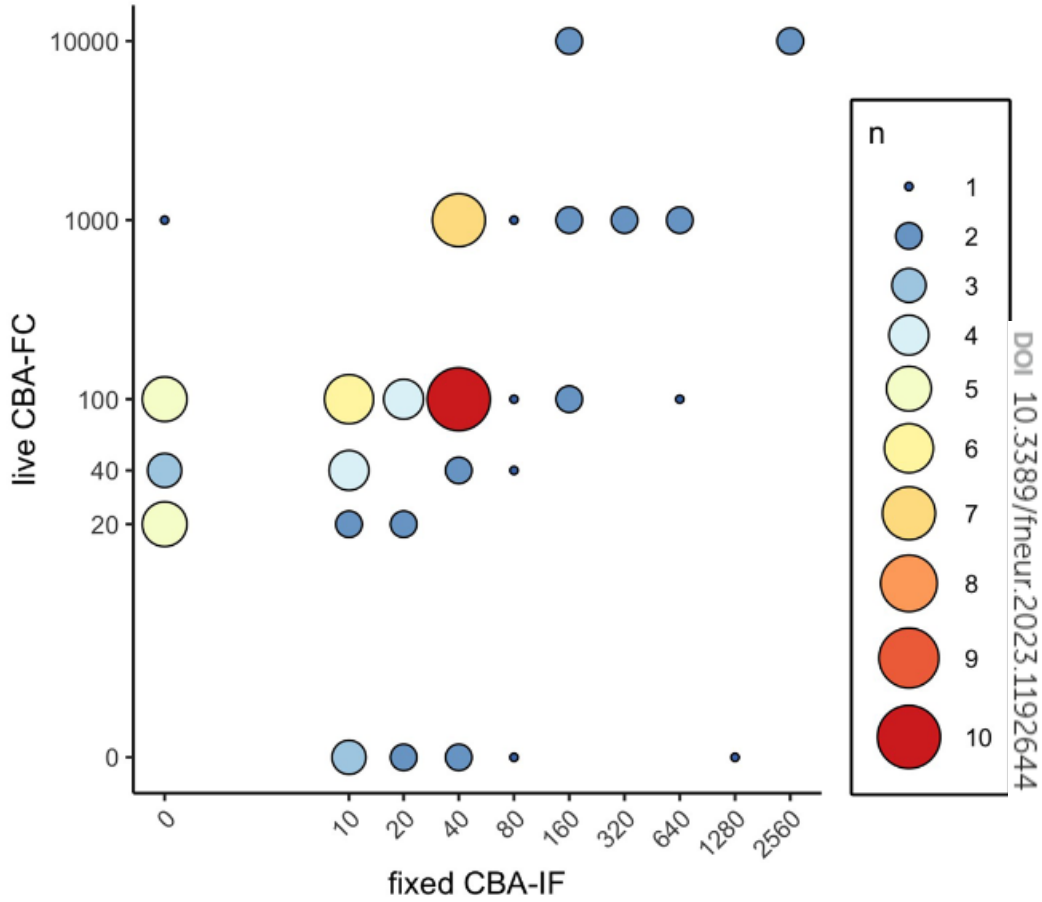
doi: 10.1001/jamaneurol.2021.0912



# Testing Methods Vary- MOG

- 93% agreement between paired live and fixed CBA for MOG (no clinical information to determine sens/spec)
- In a study of 91 clinically defined patients (LETM, ADEM, ON, seroneg NMO):
  - » 21 pos by live CBA-FC (1 FP in MS)
  - » 23 pos by fixed CBA-IF (5 FP in MS)

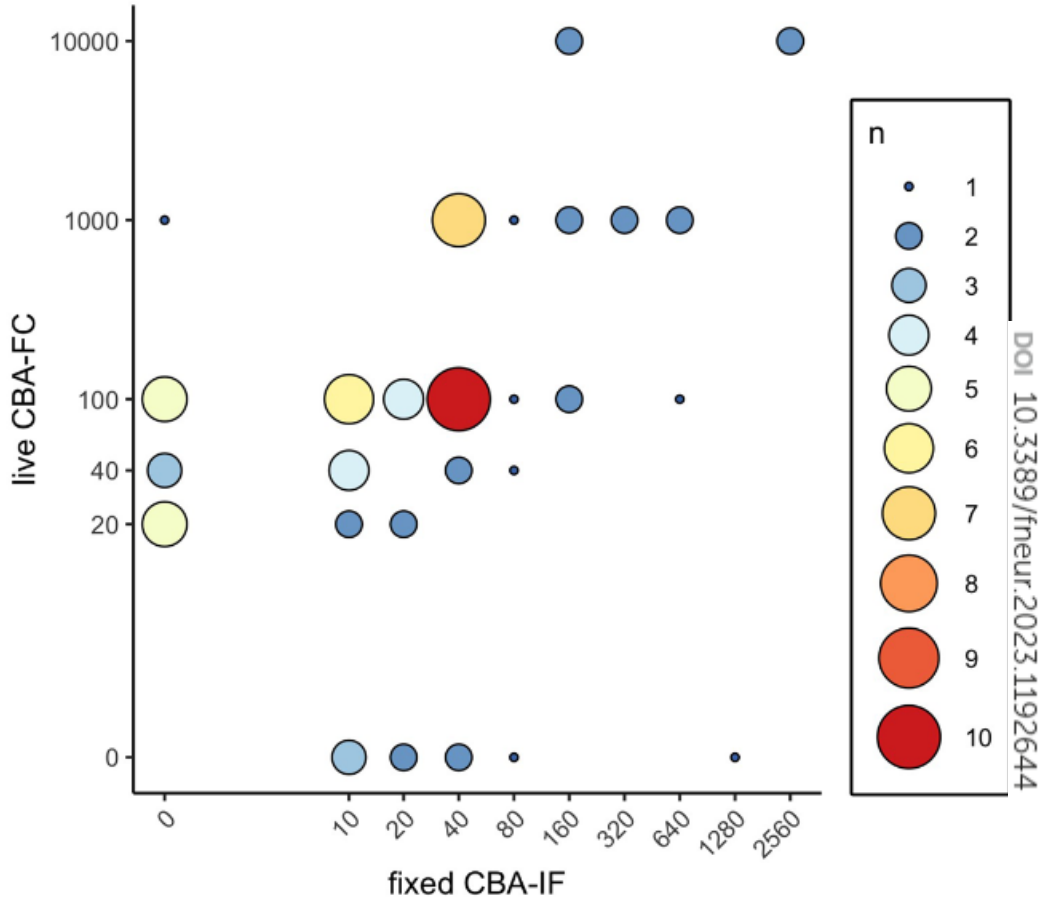
doi: 10.1212/WNL.0000000000007096




DOI 10.3389/fneur.2023.1192644

# Testing Methods Vary- MOG

- 93% agreement between paired live and fixed CBA for MOG (no clinical information to determine sens/spec)
- The clinical criteria need to be considered as well as antibody test and titer
- Note that live and fixed CBA paired samples have different titers (fixed tend to have lower titer)



# What is less muddy?

- Positive line blot testing with negative tissue immunofluorescence is of limited value
- Antibody titers do not necessarily correlate with disease severity but low titers have a lower PPV
- Unclassified antibodies remain, and clinical decisions should not be made based on antibody testing alone
- Testing in both serum and CSF is ideal for initial screening
- We have a lot to learn about improving these diagnostic tests and collaboration will be needed to give all patients access to high quality testing

# Strategies To Provide High Value Care

- Know the testing your lab offers and how it performs
- Have open lines of communication with patient-facing physicians- the lab likely knows more about this testing than they do!
- Recognize that there is a huge gap in patient-facing physician's knowledge of neural autoantibody testing, and you can help fill that



*ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.*

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