STANDARDIZATION OF IMMUNE MARKERS FOR SCREENING AND CONFIRMATION:

Islets Autoantibody Standardization Program perspective

on behalf of the IASP committee

Vito Lampasona, IRCCS San Raffaele Scientific Institute , Milano, Italy

General Population Screening for T1D,
6th Childhood Diabetes Prevention Symposium
November 9-10, 2023

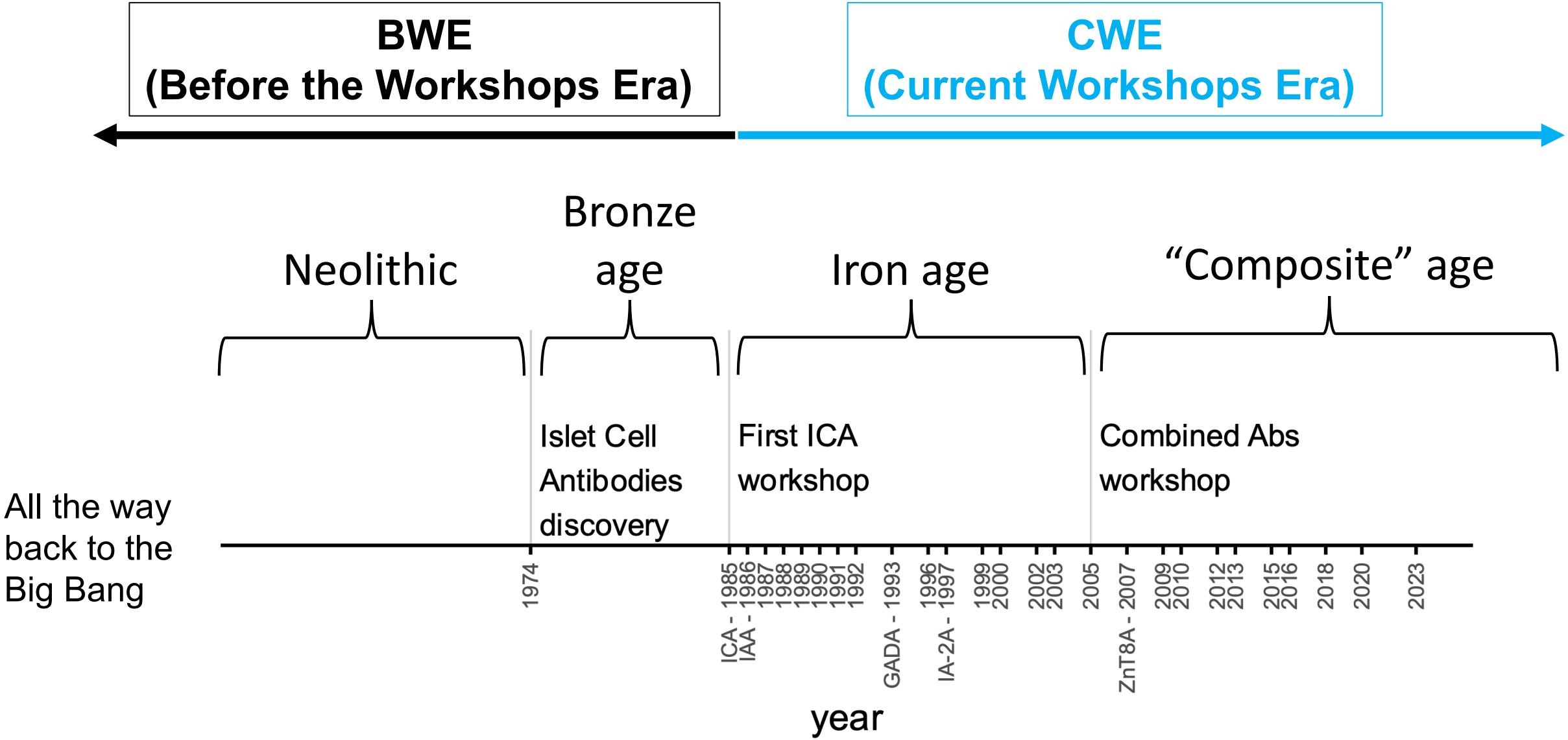
Islets Autoantibody Standardization Program (IASP)

- IASP aims at improving and standardizing the measurement of autoantibodies predictive of type 1 diabetes

How?

- IASP supports the harmonization of antibody testing across laboratories by
  - improving methods,
  - providing technical support,
  - information and training
- IASP organizes interlaboratory assay comparisons workshops
- IASP provides reference materials for the development of new measurement technologies

A IASP “perspective” on world events ..... a timeline



The current IASP committee members and sponsors

Beena
Akolkar

Peter
Achenbach

Clive
Wasserfall

Ilaria
Marzinotto

Anna
Long

David
Pittman

Vito
Lampasona

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

NIH National Institute of Diabetes and Digestive and Kidney Diseases

IDS IMMUNOLOGY OF DIABETES SOCIETY

BUT ……. lots of people contributed to the standardization of islet autoantibody assays.

Some major contributions are not reflected well in the authorship of workshop papers: first example

G.F. Bottazzo

This is the outcome of an AI prompt: Draw Bottazzo as an Italian navigator with a caravel and landing on immunostained pancreatic islets.

The outcomes is ……. Ok, let's move on

Second example:

George Eisenbarth

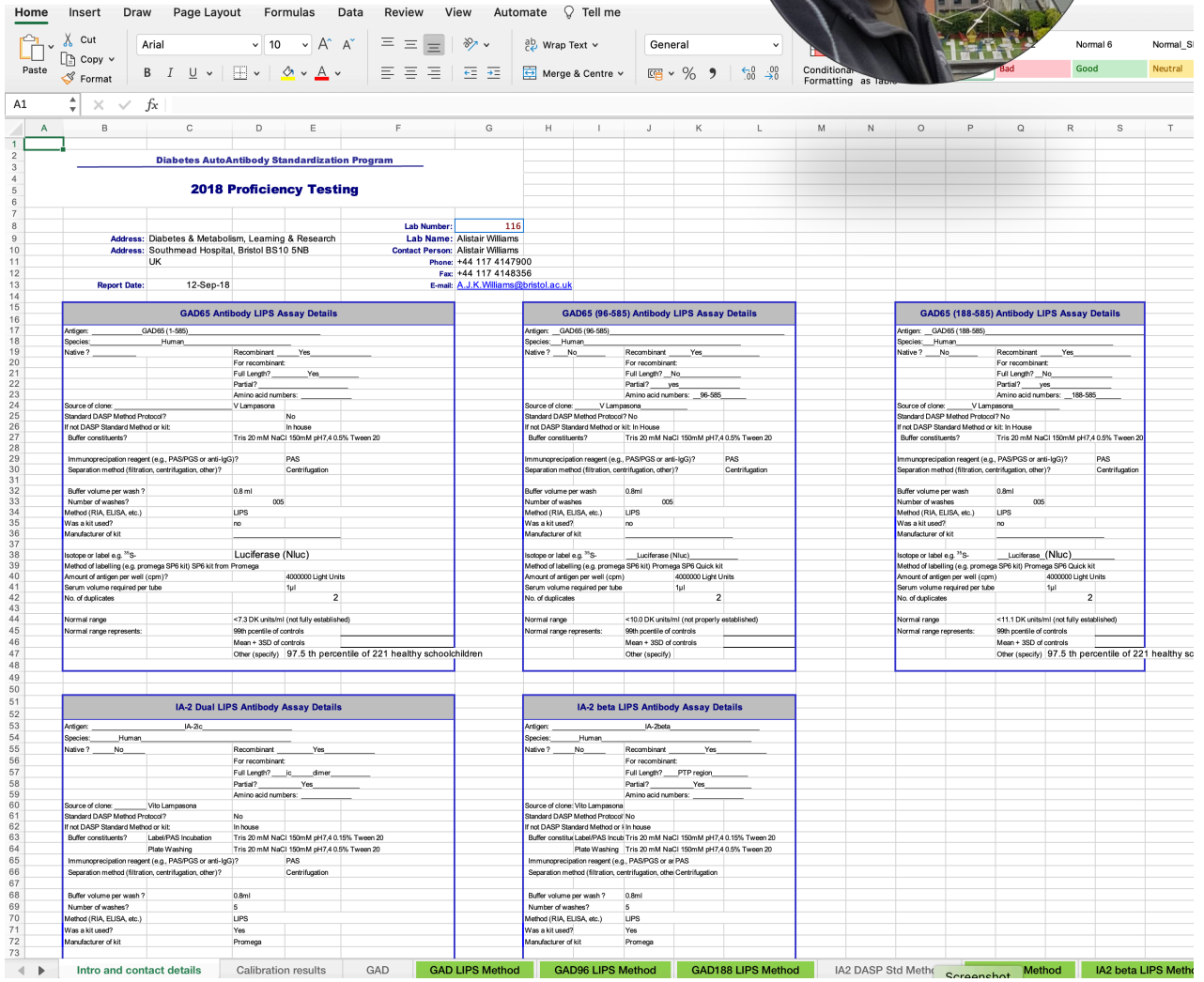
Small name in the graph, big impact in reality.
He was incredibly supportive.
It’s thanks to him that for many years the workshop repository was full of samples

IASP interlaboratory assay comparison workshops

- Every ~1.5 years
- ~150 coded sera are sent to participating laboratories (50 new onset T1D and 100 blood donor sera)
- Labs test the sera in blind using assay format of choice

Assay results are returned to the organizing committee for central analysis of assay performance

- Dave Pittman at UF non only organizes the “building” and distribution of sample sets
- He receives hundreds of submitted assay reports
- Collate the data together and create a single large dataset using macros and …..
- ….. a lot of sweat! Labs can be very creative in the way they modify our standardized reporting excel files



Diabetes AutoAntibody Standardization Program

2018 Proficiency Testing

Lab Number: 116
Address: Diabetes & Metabolism, Learning & Research
Lab Name: Alistair Williams
Address: Southmead Hospital, Bristol BS10 5NB
Contact Person: Alistair Williams
UK
Phone: +44 117 4147900
Fax: +44 117 4148356
Report Date: 12-Sep-18
E-mail: A.J.K.Williams@bristol.ac.uk

GAD65 Antibody LIPS Assay Details

GAD65 (96-585) Antibody LIPS Assay Details

GAD65 (188-585) Antibody LIPS Assay Details

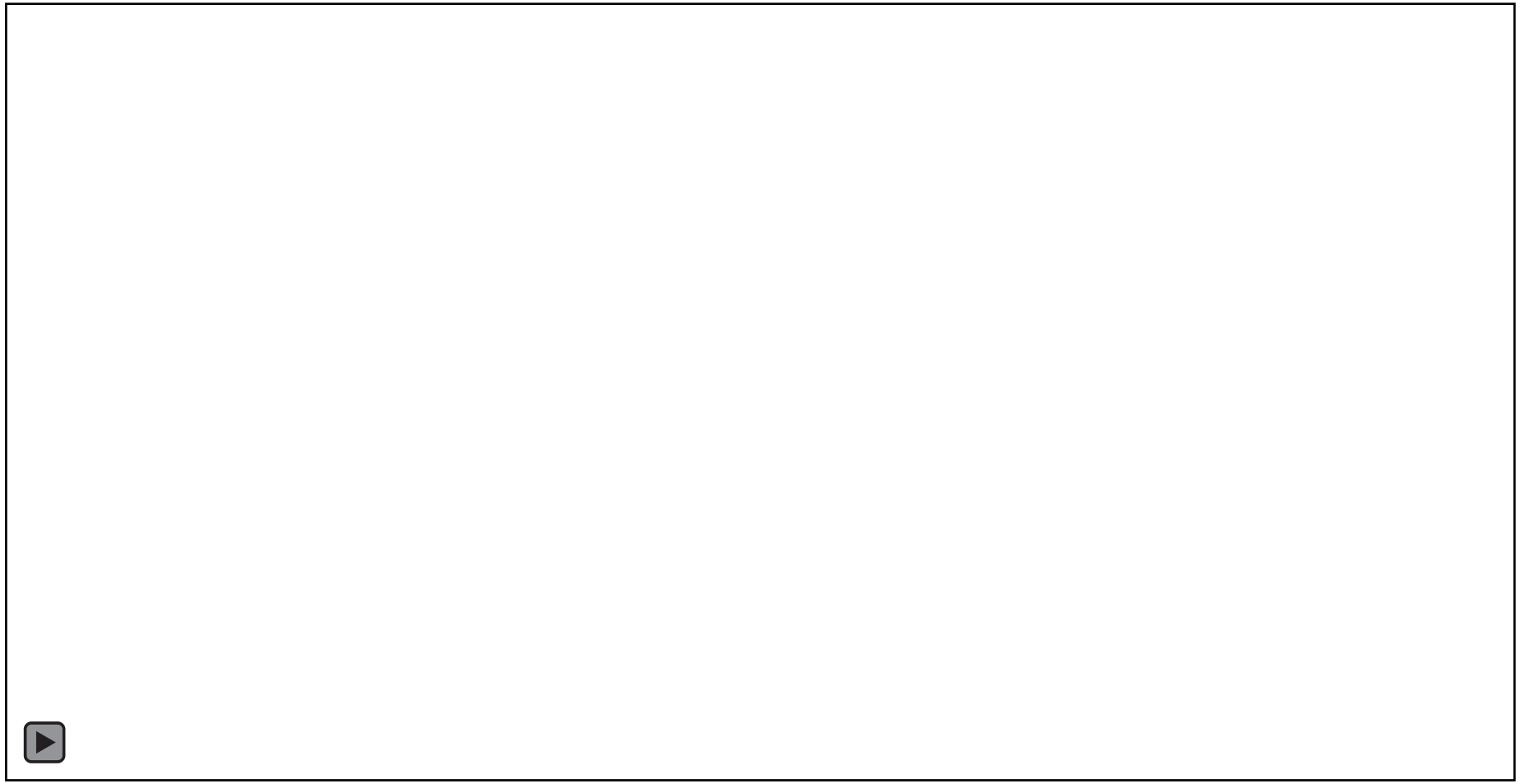
IA-2 Dual LIPS Antibody Assay Details

IA-2 beta LIPS Antibody Assay Details

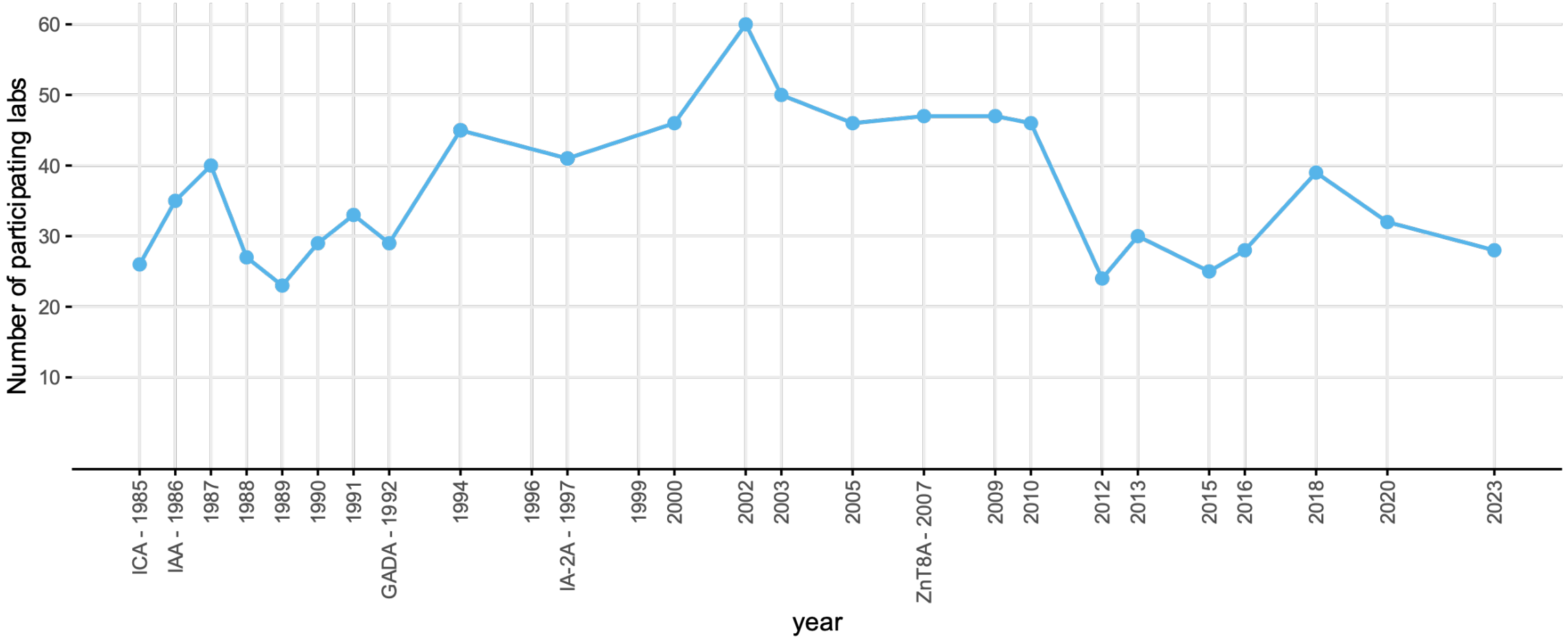
Intro and contact details | Calibration results | GAD | GAD LIPS Method | GAD96 LIPS Method | GAD188 LIPS Method | IA2 DASP Std Meth | Screenshot | Method | IA2 beta LIPS Meth

We then move to R for summary tables, additional statistics, graphs. Our analyses “documentation” is in R markdown notebooks

- The concept is inspired by the principles of reproducible science
- The goal is to document everything we do for future reference



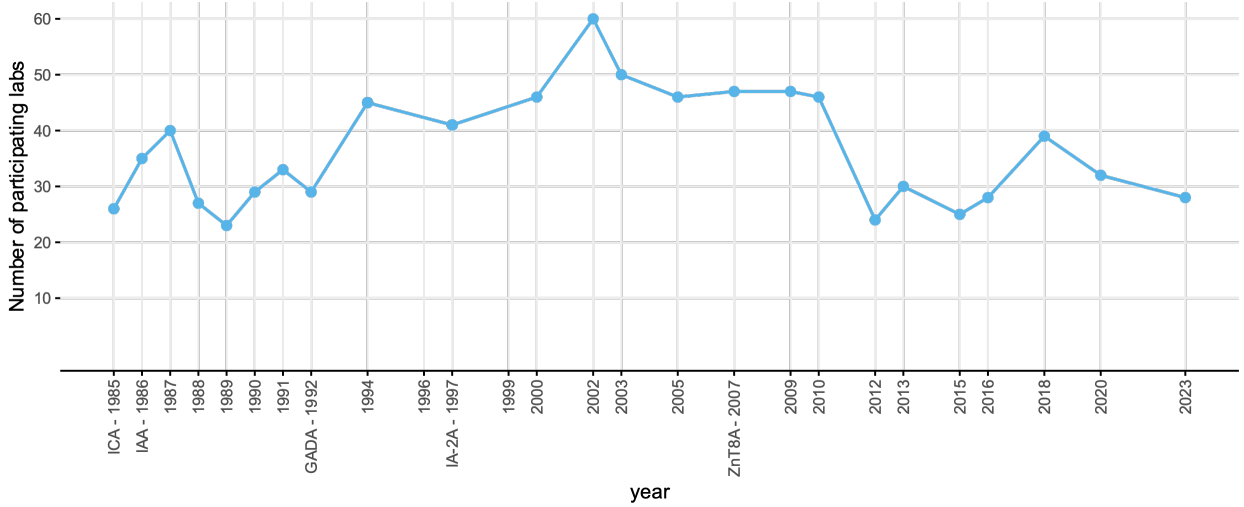
How many labs participate in IASP workshops?
It varies, over the last few years we hover around 30



First IASP perspective on large scale screenings

- IF the T1D autoantibody screening is going to be performed by a much larger number of labs then …….
- Changes are required either for the resources allocated to the workshop or to its format (likely both)

Another IASP perspective: how representative the participating labs are of all labs doing T1D testing?

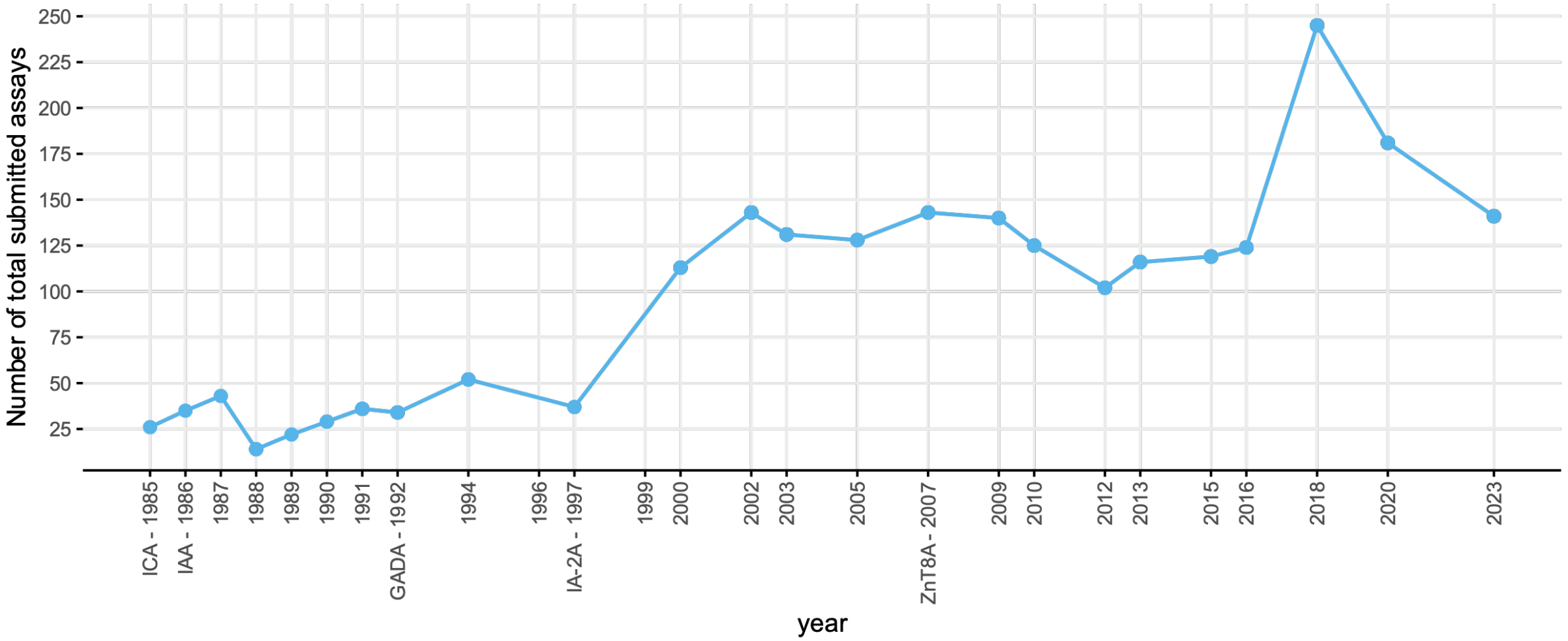


- In truth they are not that many, and are mostly research labs
- Several are very experienced (~1/3 participated in all workshops!!?!?!!)
- Some labs also do also routine diagnostics BUT ….. the vast majority of routine labs measuring autoAbs out there in the big world never/ever participated in workshops
- It is doubtful that the performance of these labs is truly representative of that in the “wild”, most diagnostics labs at best test very few quality control samples each year

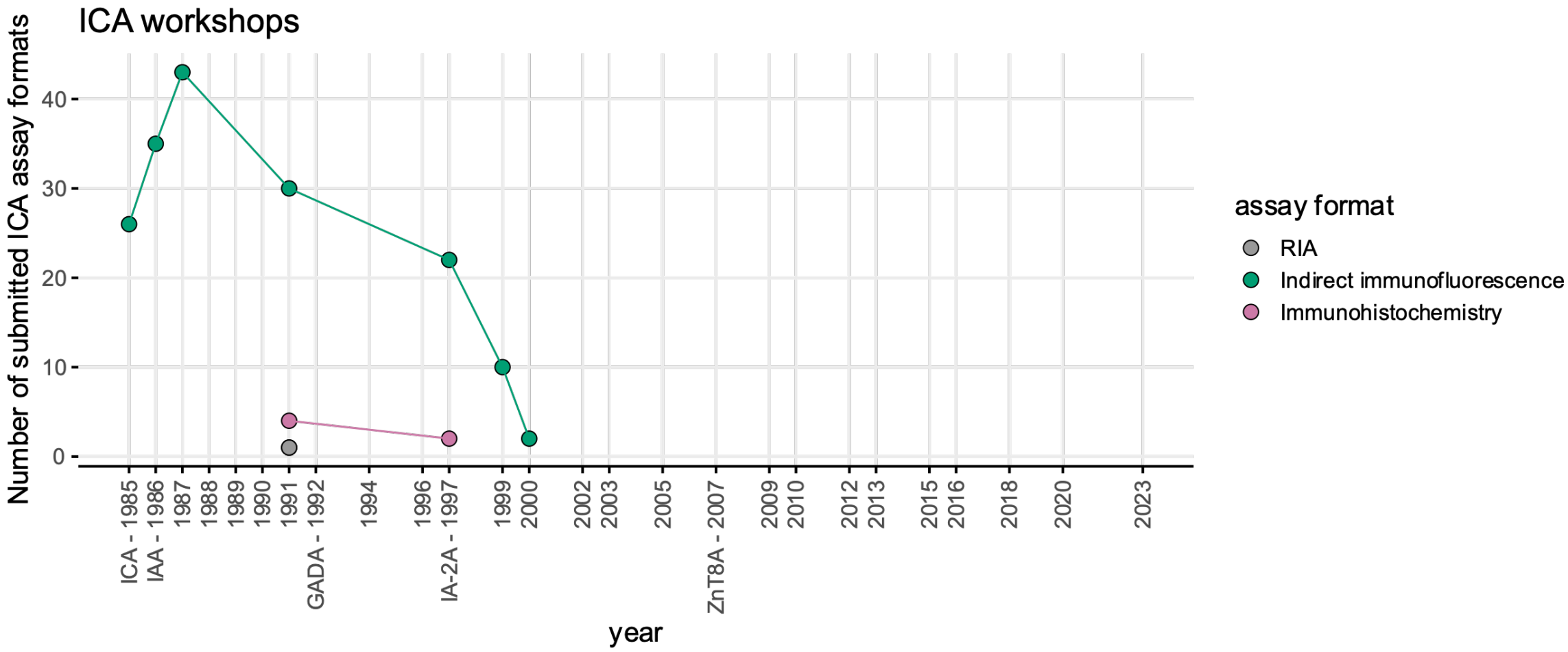
Second IASP perspective on large scale screenings

- Sera included in the workshop are not from at-risk individuals or children from the general population
- Serum samples are obtained by venipuncture while current screenings are based on capillary blood samples
- A bit of caution is needed in extrapolating the performance of assays in the IASP setting to that in a population screening

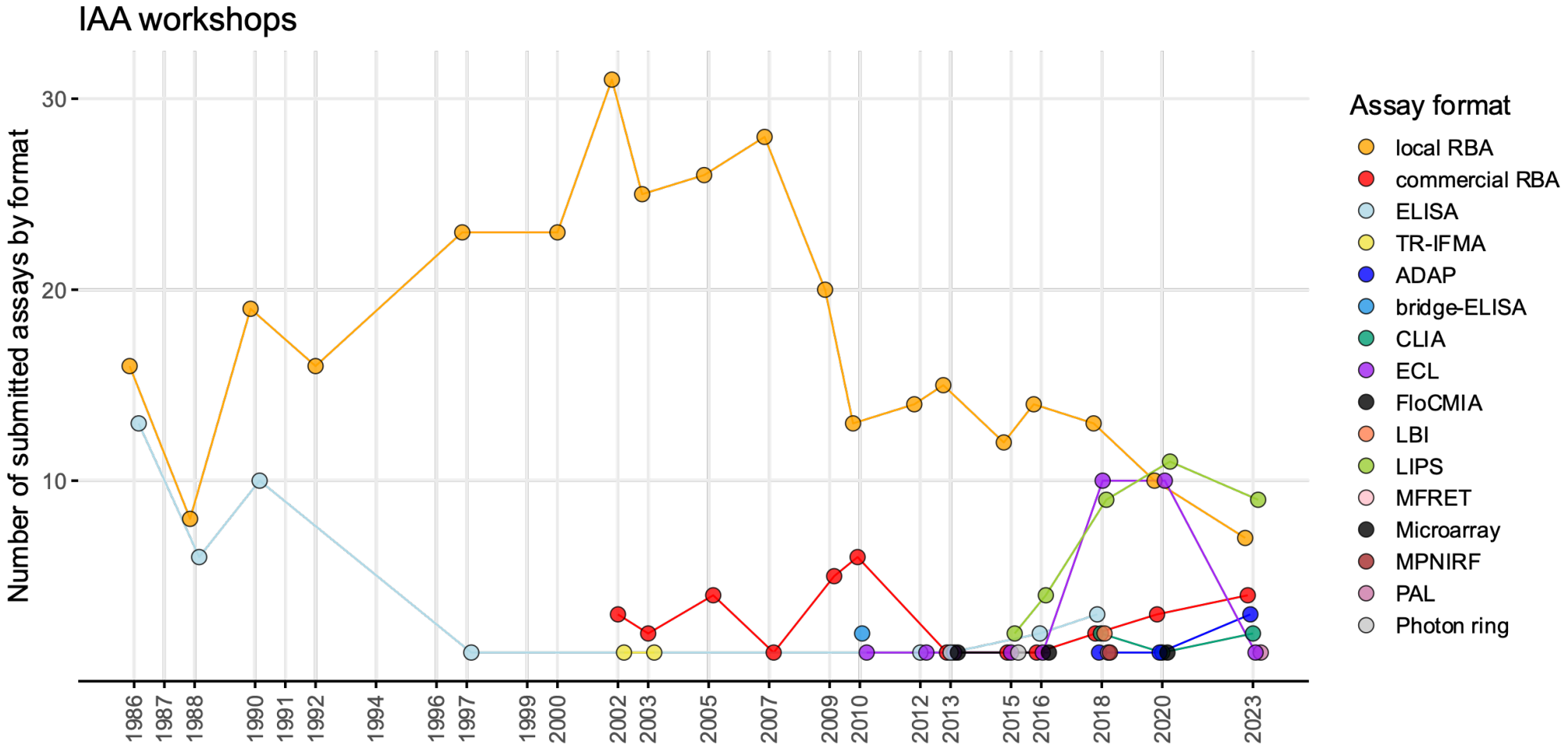
Back to the workshops: submitted assays number is much higher, on average more than a 100.



Type of submitted assays/formats changed over time: ICA assays were once dominant and then “crashed”



IAA assays tell a different story: prolonged predominance of the Radio Binding Assay (RBA) format

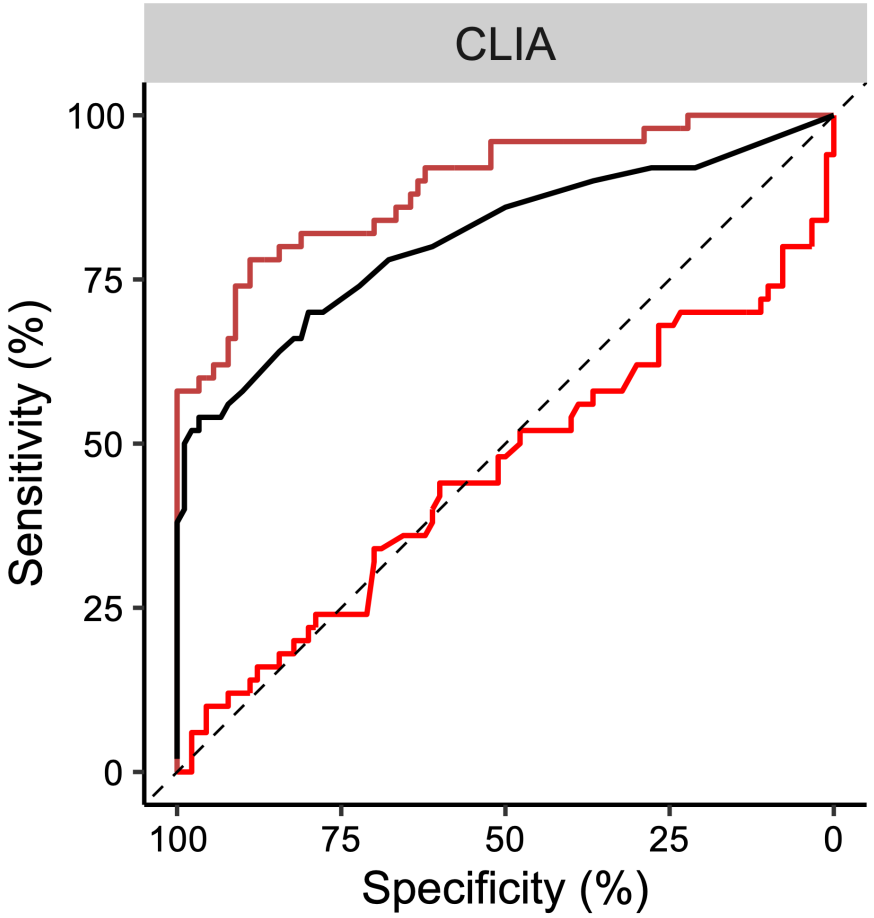


In recent years different formats are finding their pace:
ECL, LIPS, ADAP, CLIA (in chronological order :-)

IASP perspective: many different assays/formats for T1D autoantibodies were submitted over time …… AND FAILED

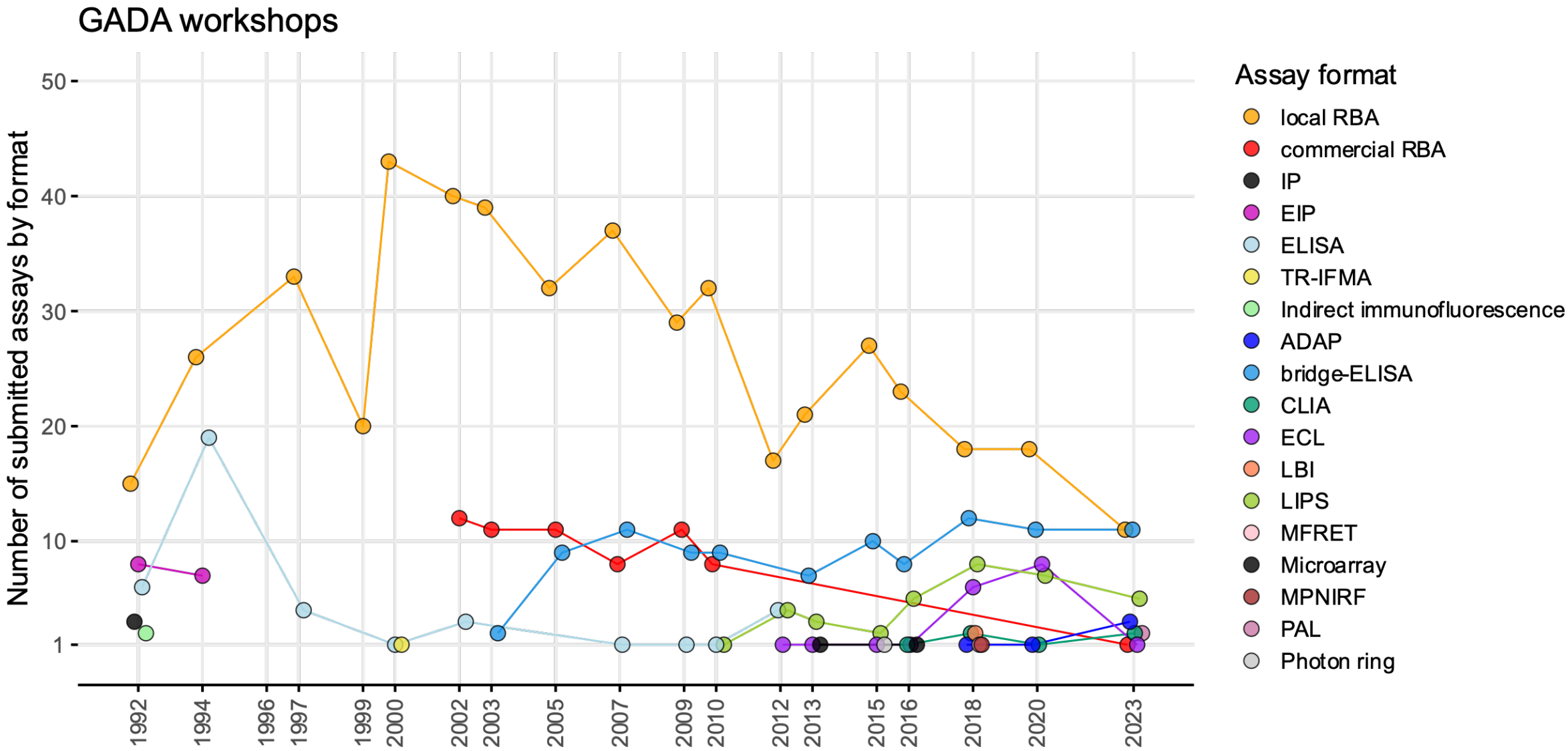
We still need IASP workshops.

- Even in the year 2023 !
- Only one of the two submitted CLIA IAA assays worked!
- It's very useful for the community that even poor assays are submitted to the workshop
- We need to know/understand what works and what doesn't.

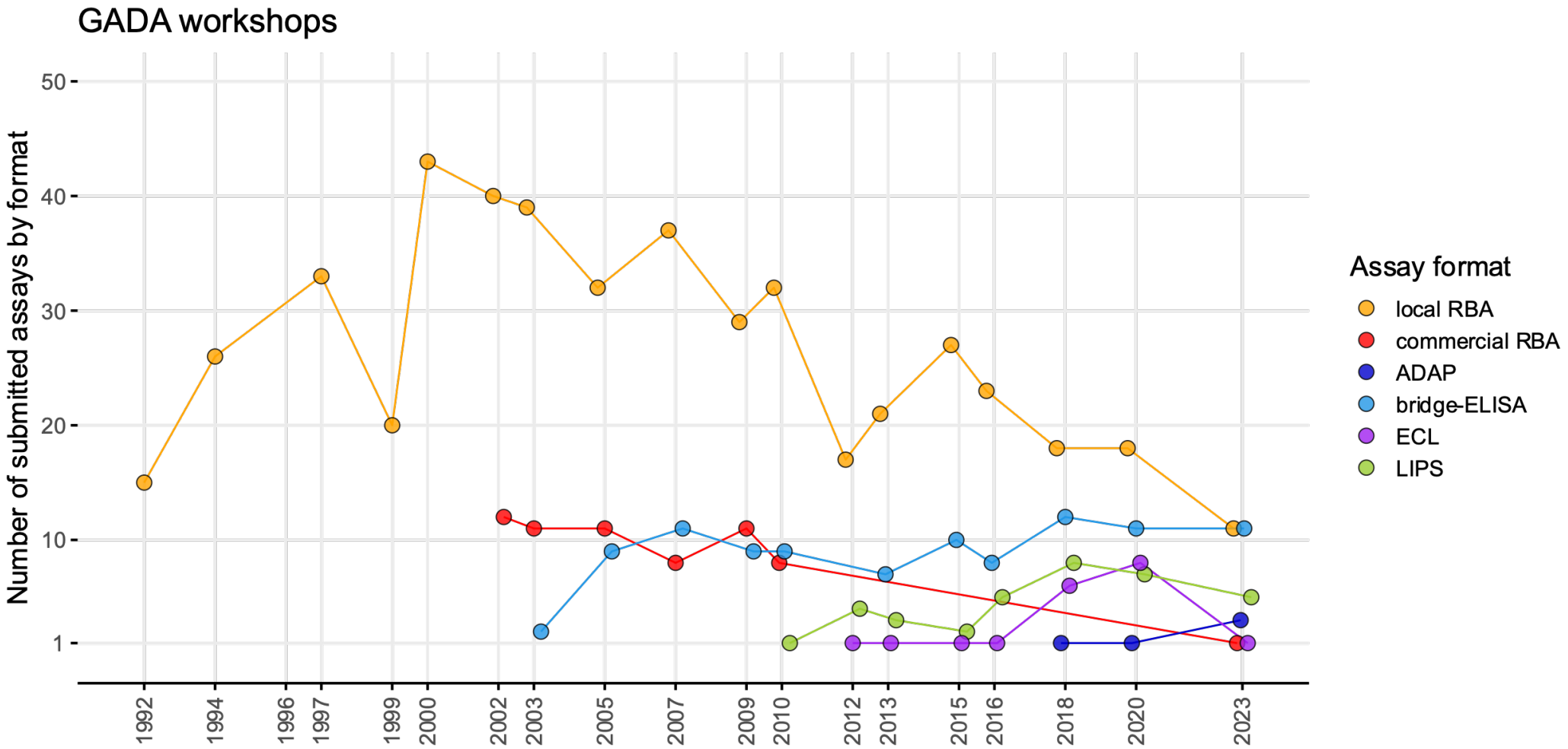


The black line corresponds to the median ROC curve calculated across all IAA assays submitted to the workshop. The dashed line is the identity line

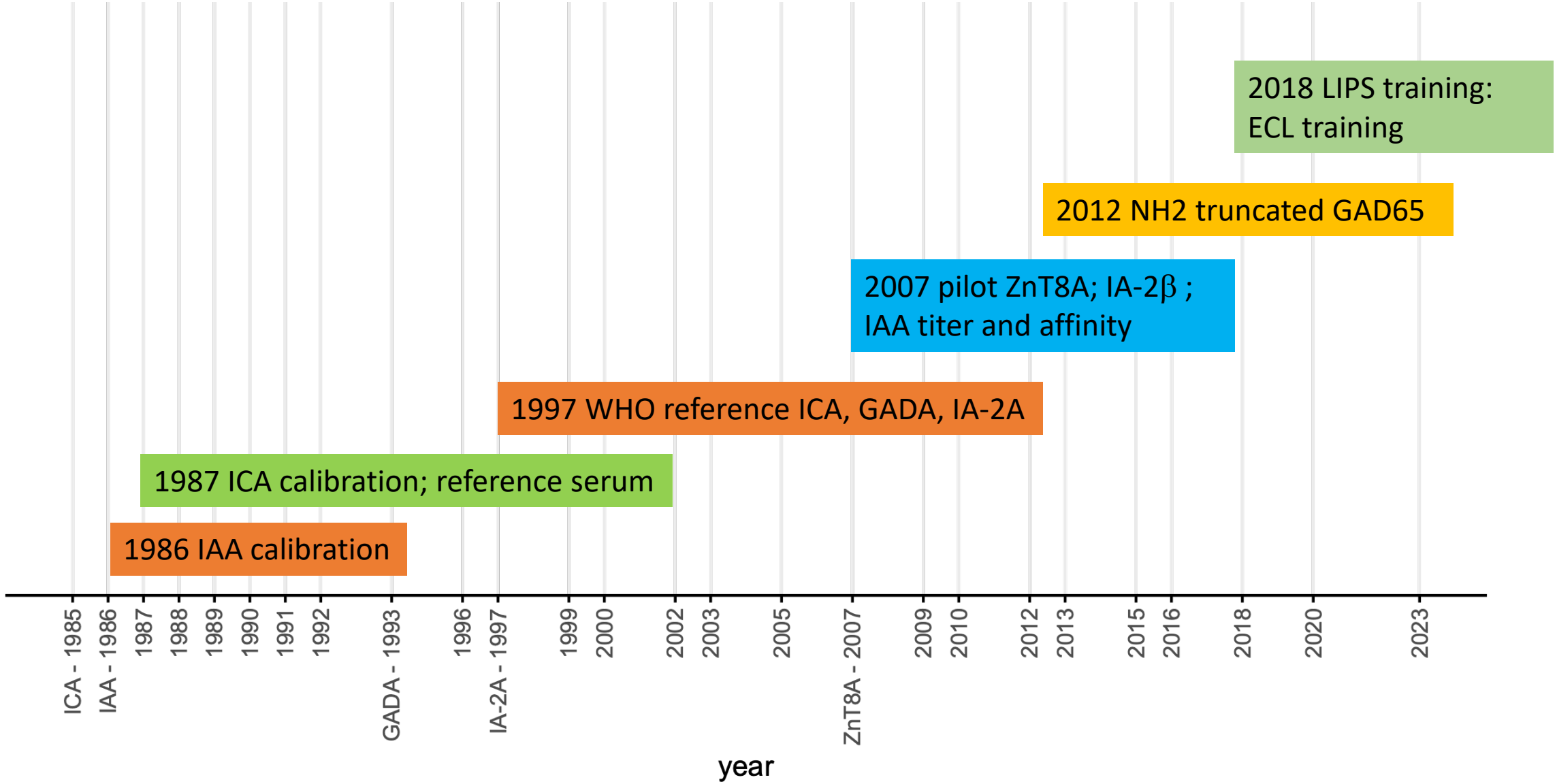
GADA assays show a somewhat similar trajectory with RBA dominating for many years



However, beginning with bridge-ELISA assays in 2003 several non-radioisotopic assay formats matched or exceeded RBA performance: Bridge-ELISA, LIPS, ECL, ADAP



There is more to the workshop: substudies



The IASP perspective on reference standards for T1D autoAbs: not quite there yet ☹

What we hoped for

- Following the 2000 workshop it was recommended the introduction of common WHO units for GADA and IA-2A.

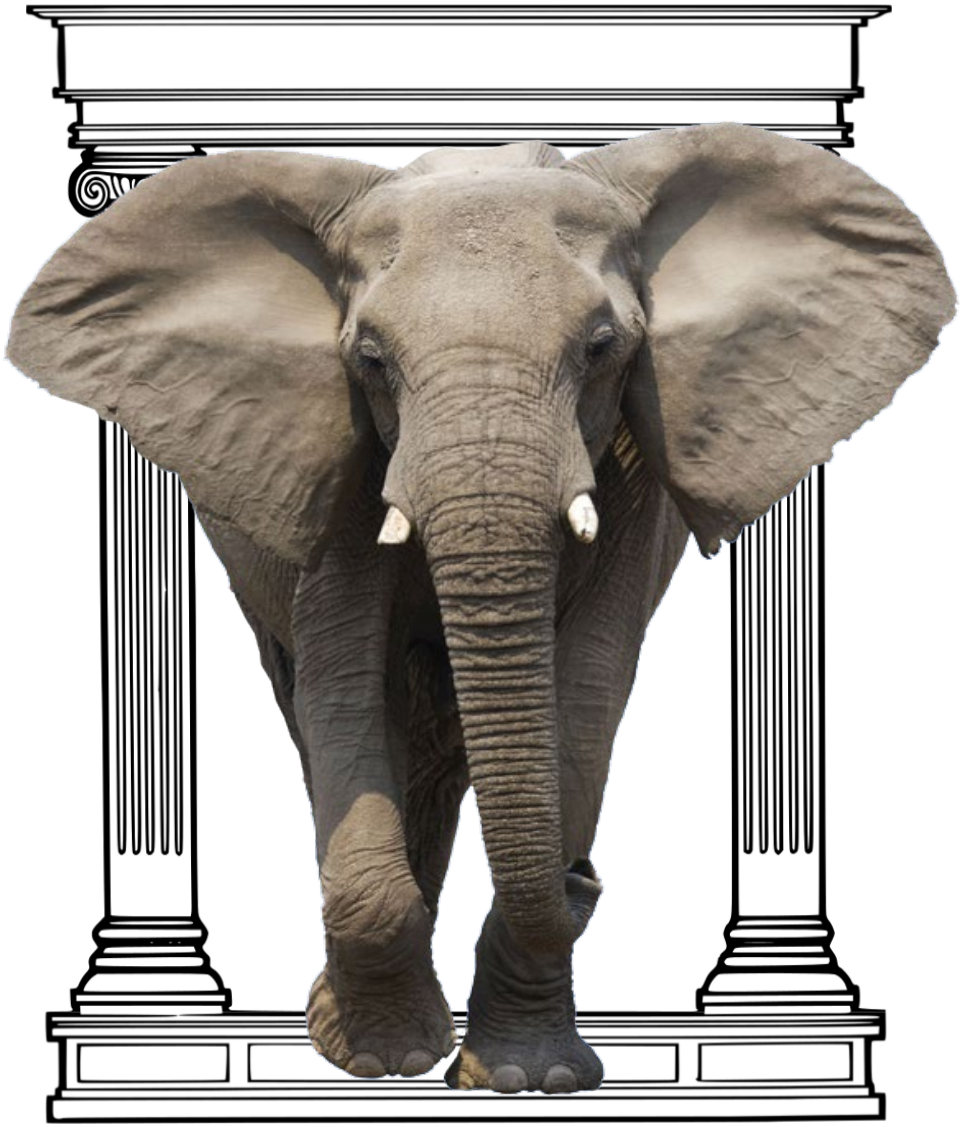
What happened

- Most labs still use “local” units
- Reference standards are missing for IAA and ZnT8A

It’s worth to keep in mind that there is no easy workaround for the production of T1D autoAb standards

Assays measuring host antibodies are best classified as quasi-quantitative

Mire-Sluis, A.R.et al. 2004. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. Journal of Immunological Methods 289, 1–16. *https://doi.org/10.1016/j.jim.2004.06.002*

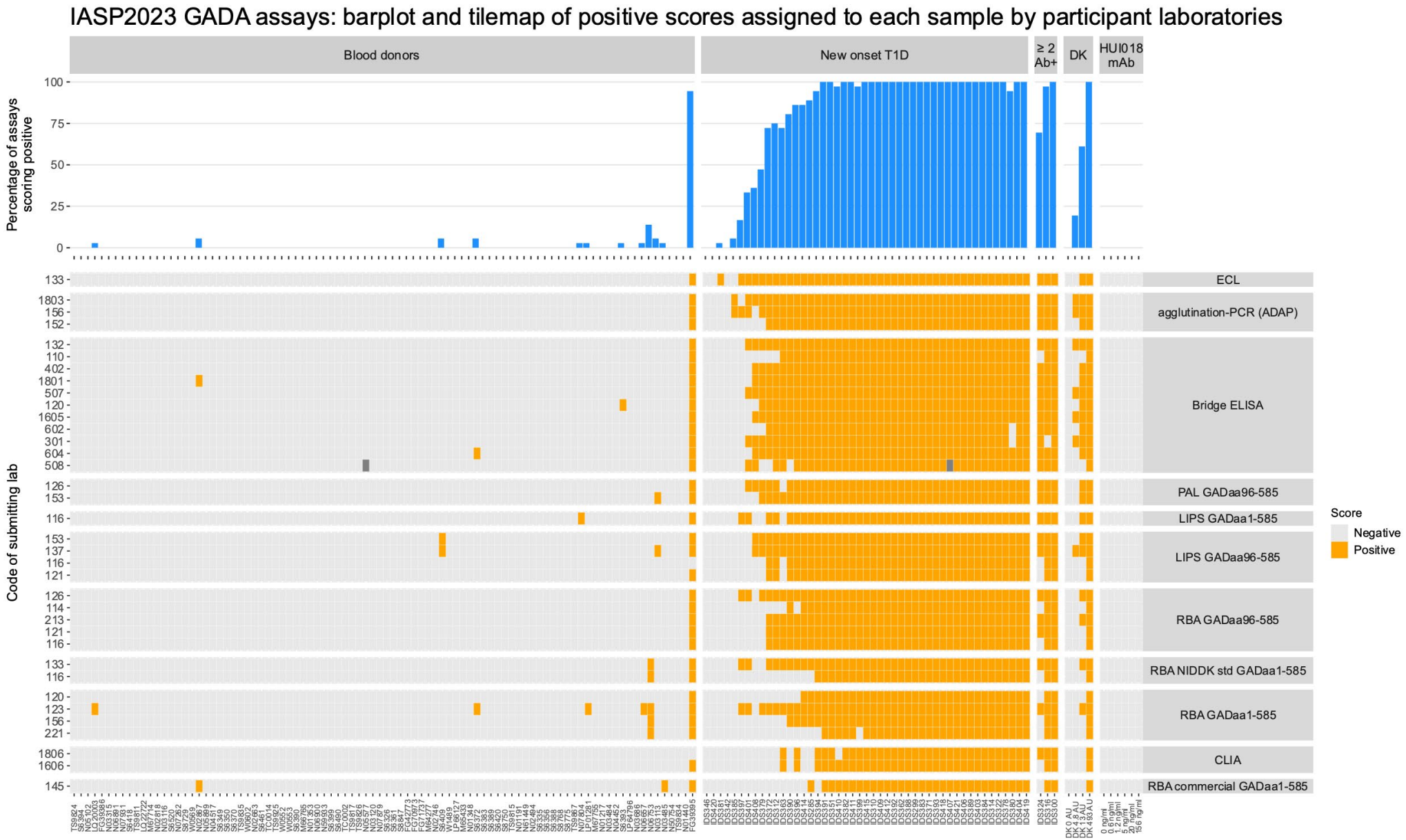


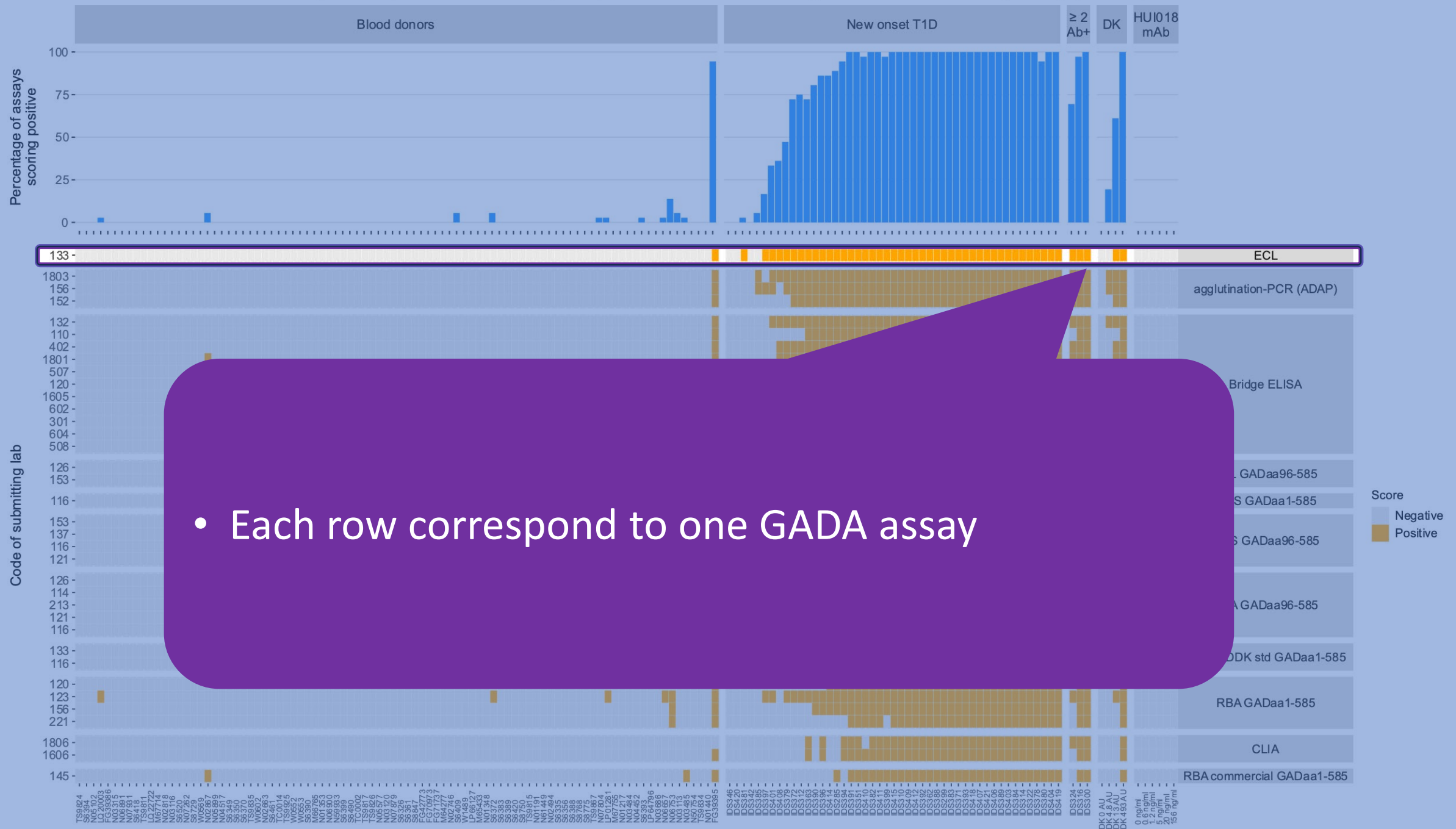
Why Quasi-Quantitative?

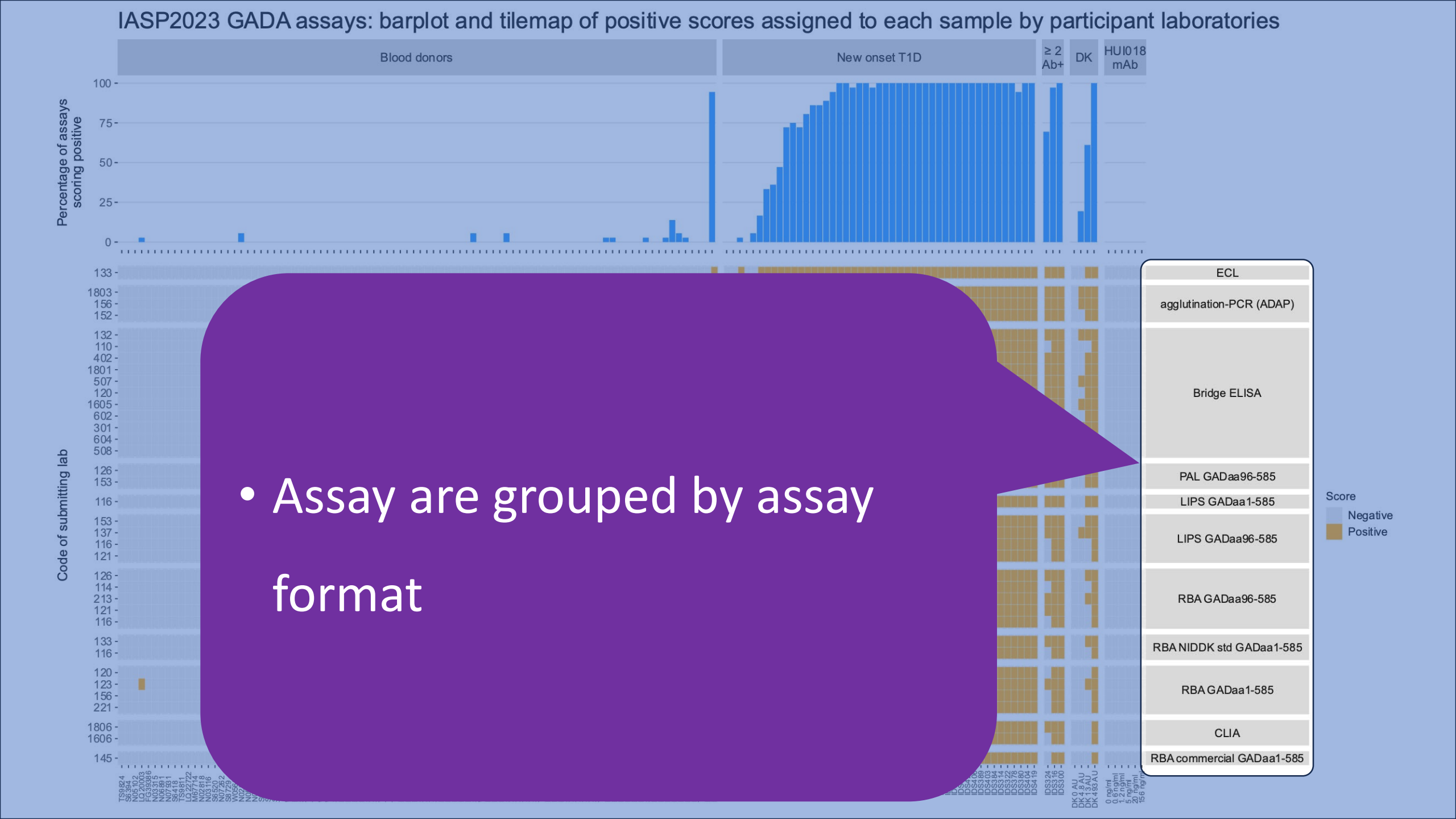
- "Due to the lack of similarity between the standard samples and test samples."
- "The reference standards may not accurately reflect the antibody affinities, proportions and other conditions in the test sample."
- "The analytes in test samples are not similar amongst themselves."
- "Since the lack of similarity implies non-parallelism, the analytical results determined from dose interpolation (calibration) from the standard curve in the absence of parallelism will represent an inexact approximation."

Mire-Sluis, A.R.et al. 2004.

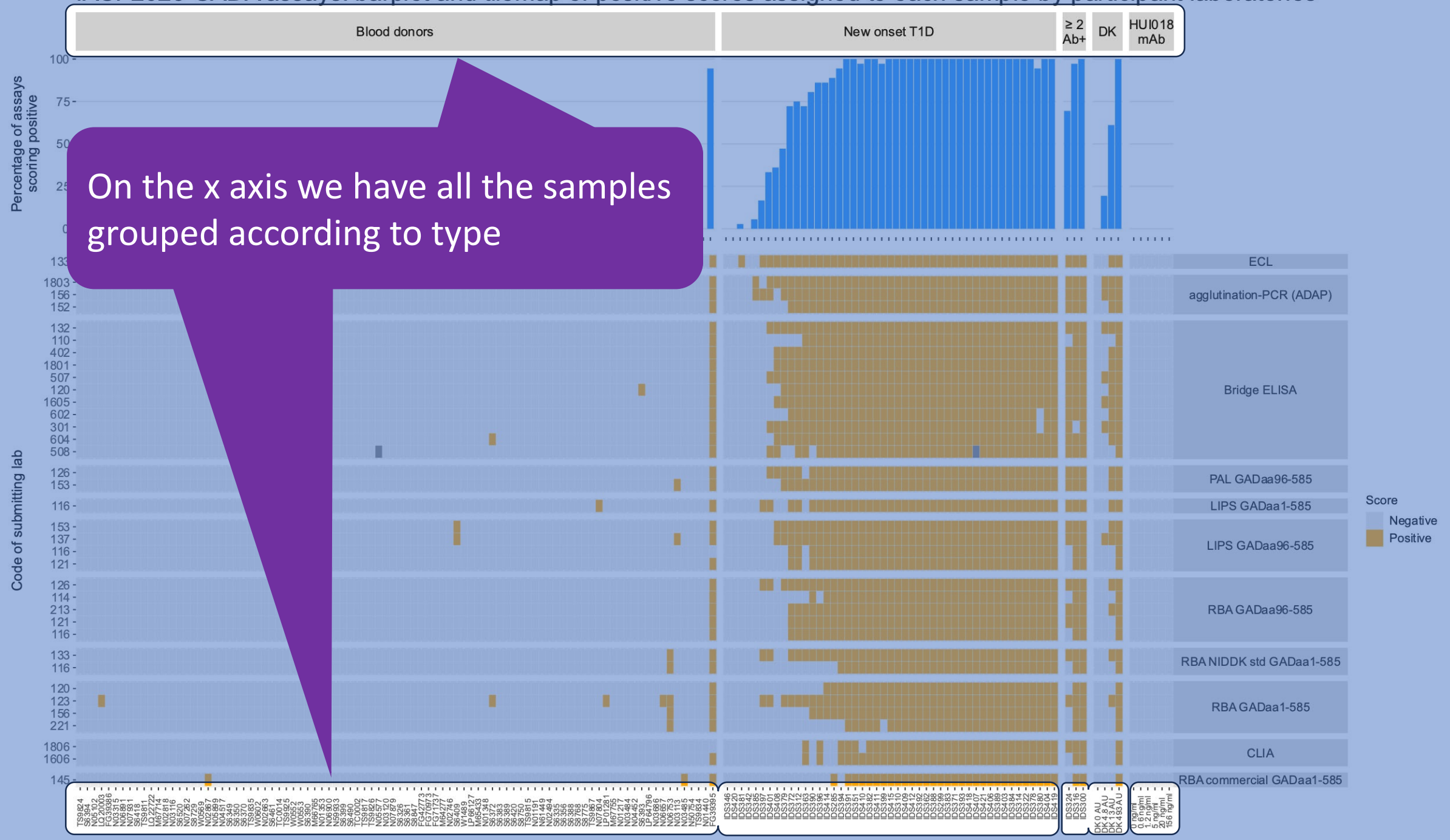
Back to workshops: assay concordance, an example for GADA

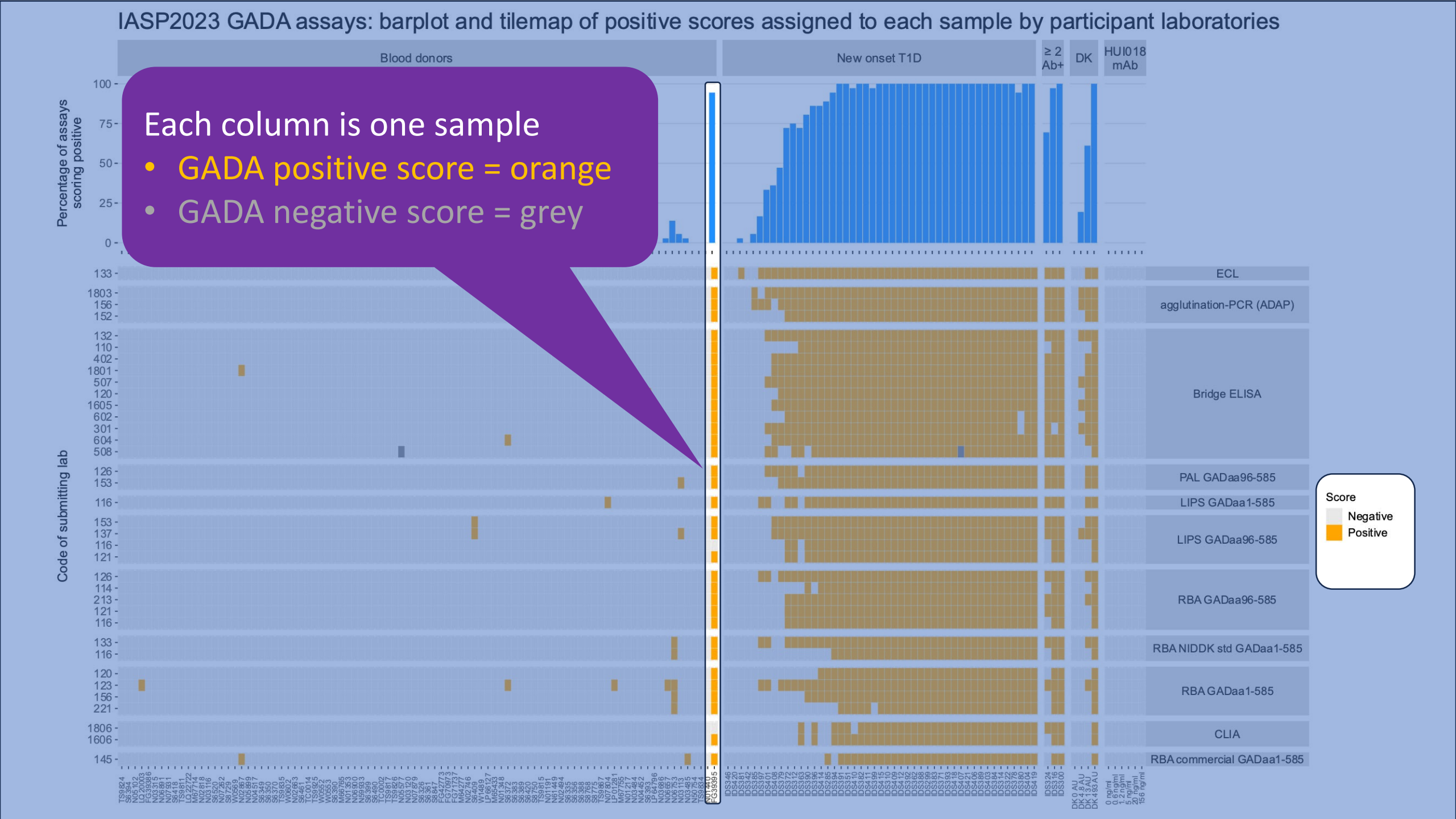



IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories
Blood donors
New onset T1D
≥ 2 Ab+
DK
HUI018 mAb
Percentage of assays scoring positive
100
75
50
25
0
133
ECL
1803
156
152
agglutination-PCR (ADAP)
132
110
402
1801
507
120
1605
602
301
604
508
Bridge ELISA
126
153
GADaa96-585
116
S GADaa1-585
153
137
116
121
S GADaa96-585
126
114
213
121
116
A GADaa96-585
133
116
DDK std GADaa1-585
120
123
156
221
RBA GADaa1-585
1806
1606
CLIA
145
RBA commercial GADaa1-585
Code of submitting lab
Score
Negative
Positive
• Each row correspond to one GADA assay

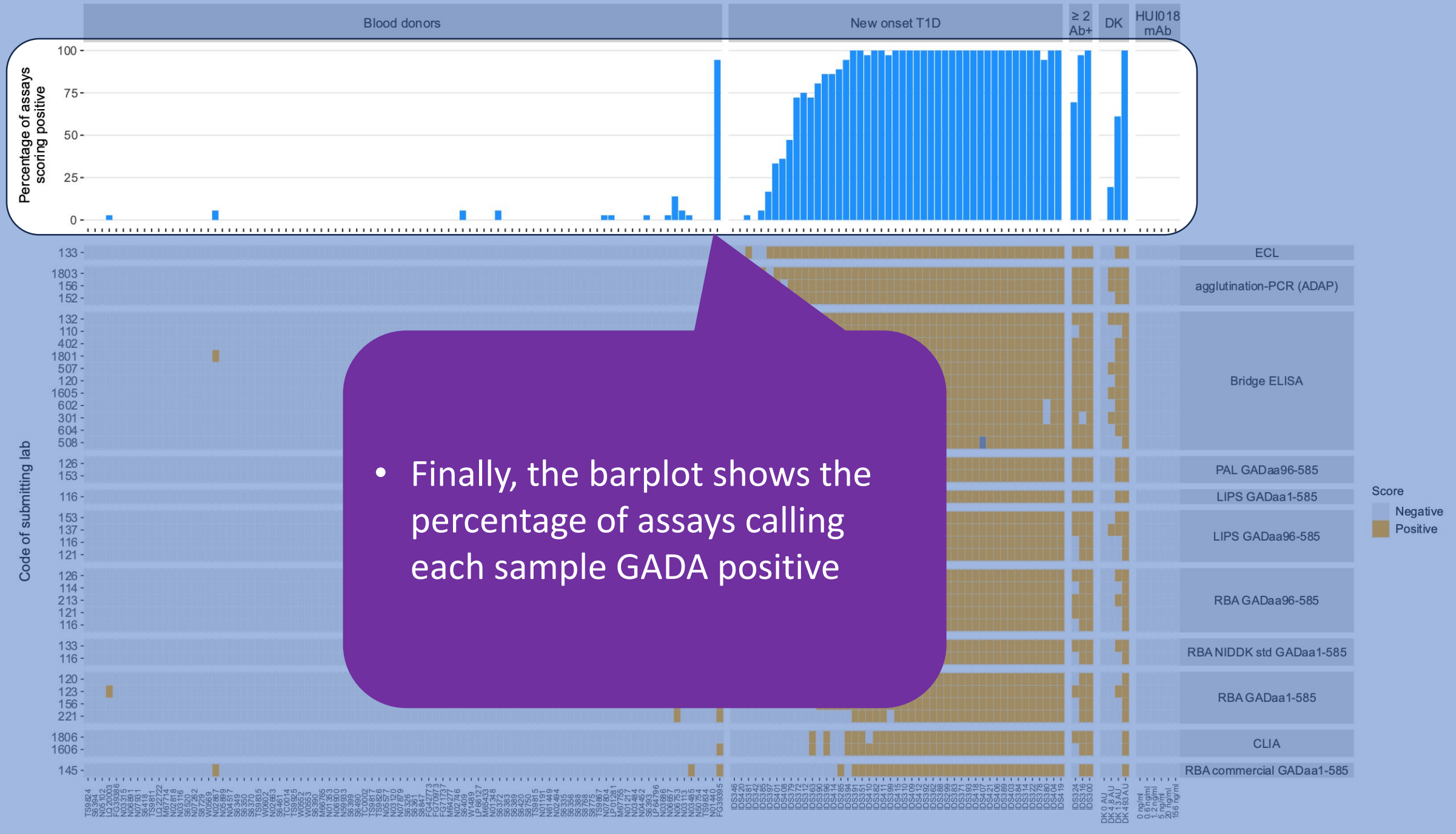

IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories
Blood donors
New onset T1D
≥ 2 Ab+
DK
HUI018 mAb
Percentage of assays scoring positive
100
75
50
25
0
Code of submitting lab
ECL
agglutination-PCR (ADAP)
Bridge ELISA
PAL GADaa96-585
LIPS GADaa1-585
LIPS GADaa96-585
RBA GADaa96-585
RBA NIDDK std GADaa1-585
RBA GADaa1-585
CLIA
RBA commercial GADaa1-585
Score
Negative
Positive
• Assay are grouped by assay format

IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories

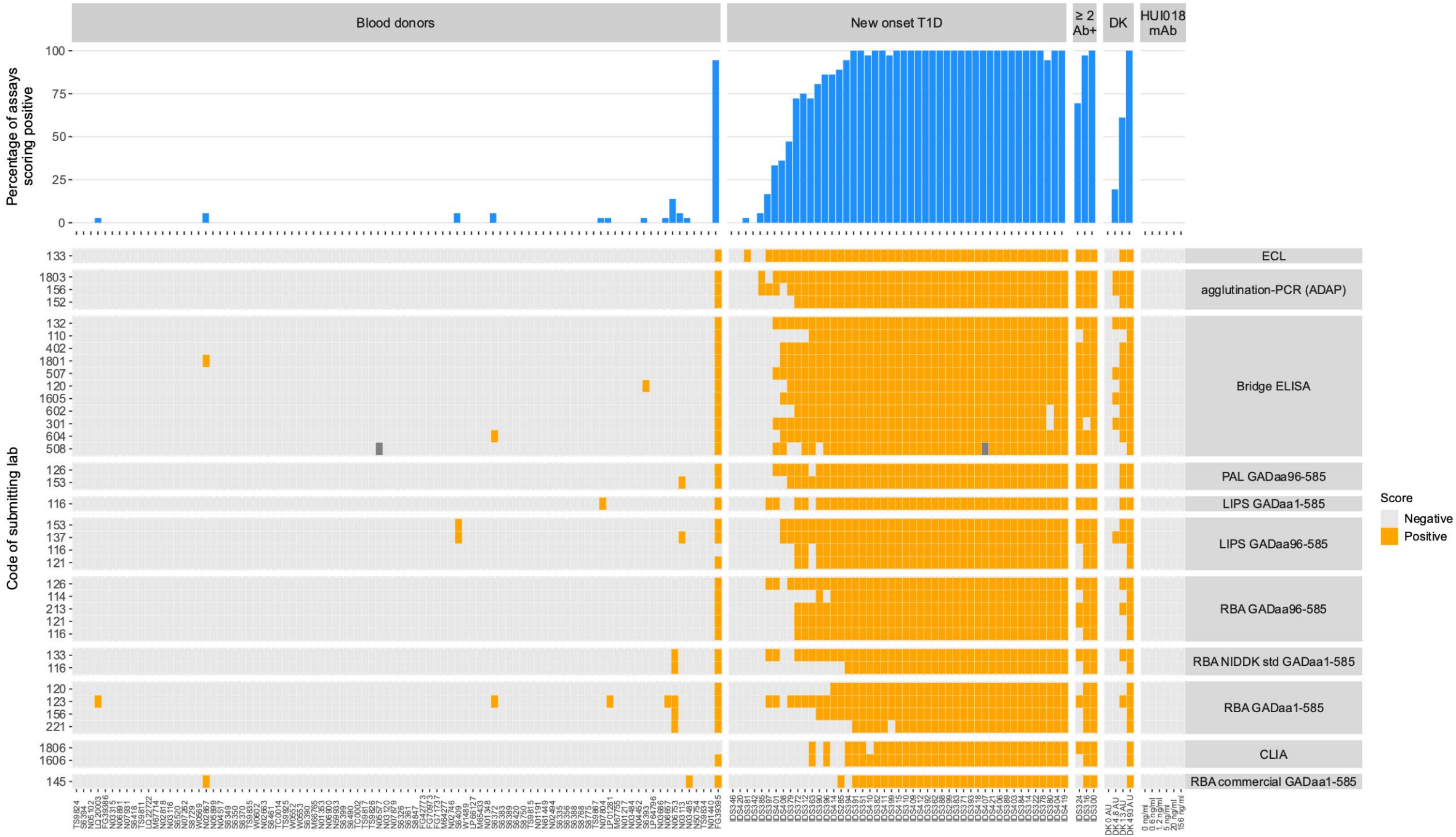


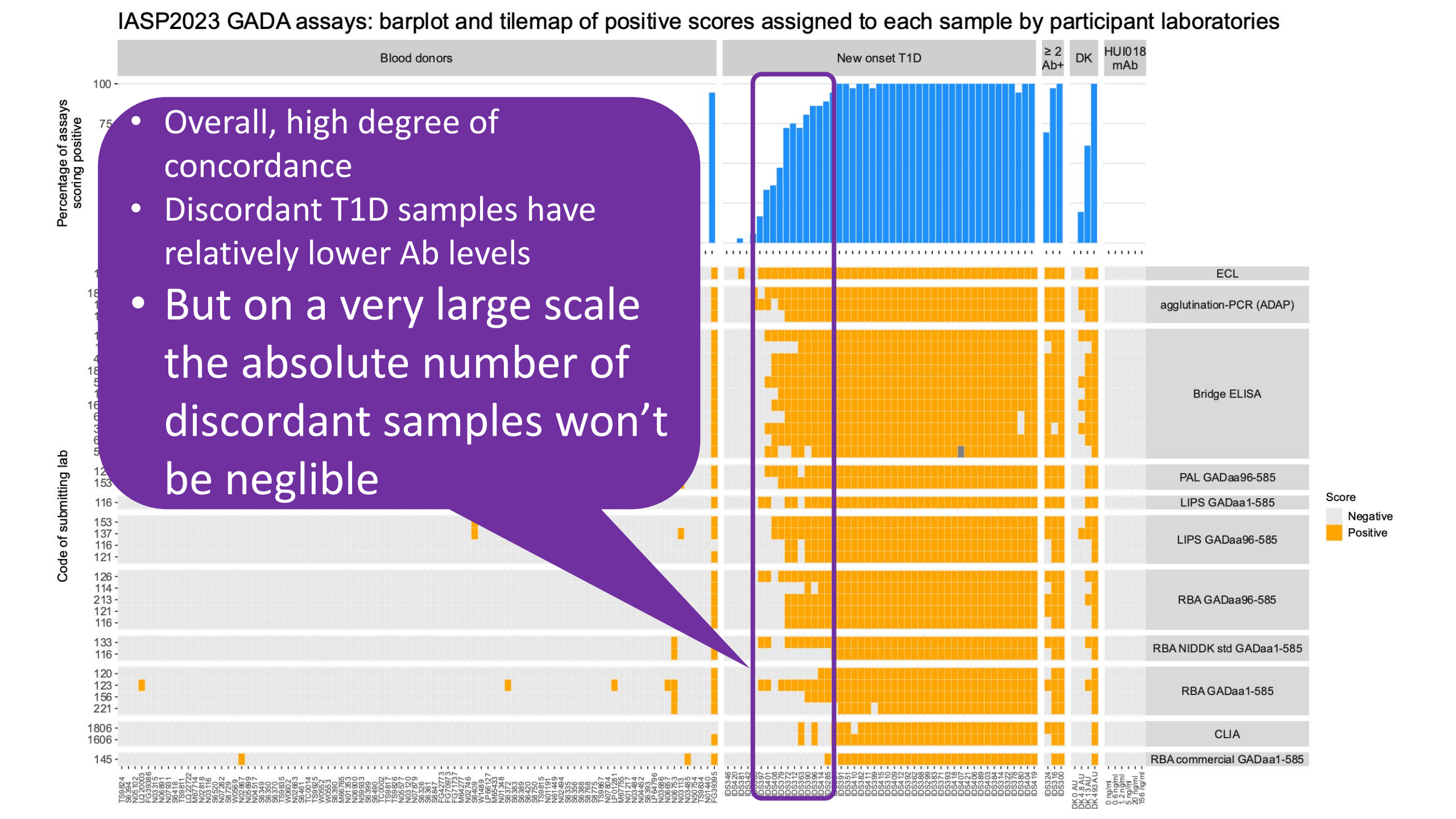

IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories
Blood donors
New onset T1D
≥ 2 Ab+
DK
HUI018 mAb
Percentage of assays scoring positive
100
75
50
25
0
Each column is one sample
• GADA positive score = orange
• GADA negative score = grey
Code of submitting lab
ECL
agglutination-PCR (ADAP)
Bridge ELISA
PAL GADaa96-585
LIPS GADaa1-585
LIPS GADaa96-585
RBA GADaa96-585
RBA NIDDK std GADaa1-585
RBA GADaa1-585
CLIA
RBA commercial GADaa1-585
Score
Negative
Positive
DK 0 AU
DK 4.8 AU
DK 13 AU
DK 493 AU
0 ng/ml
1.25 ng/ml
2.5 ng/ml
5 ng/ml
20 ng/ml
156 ng/ml

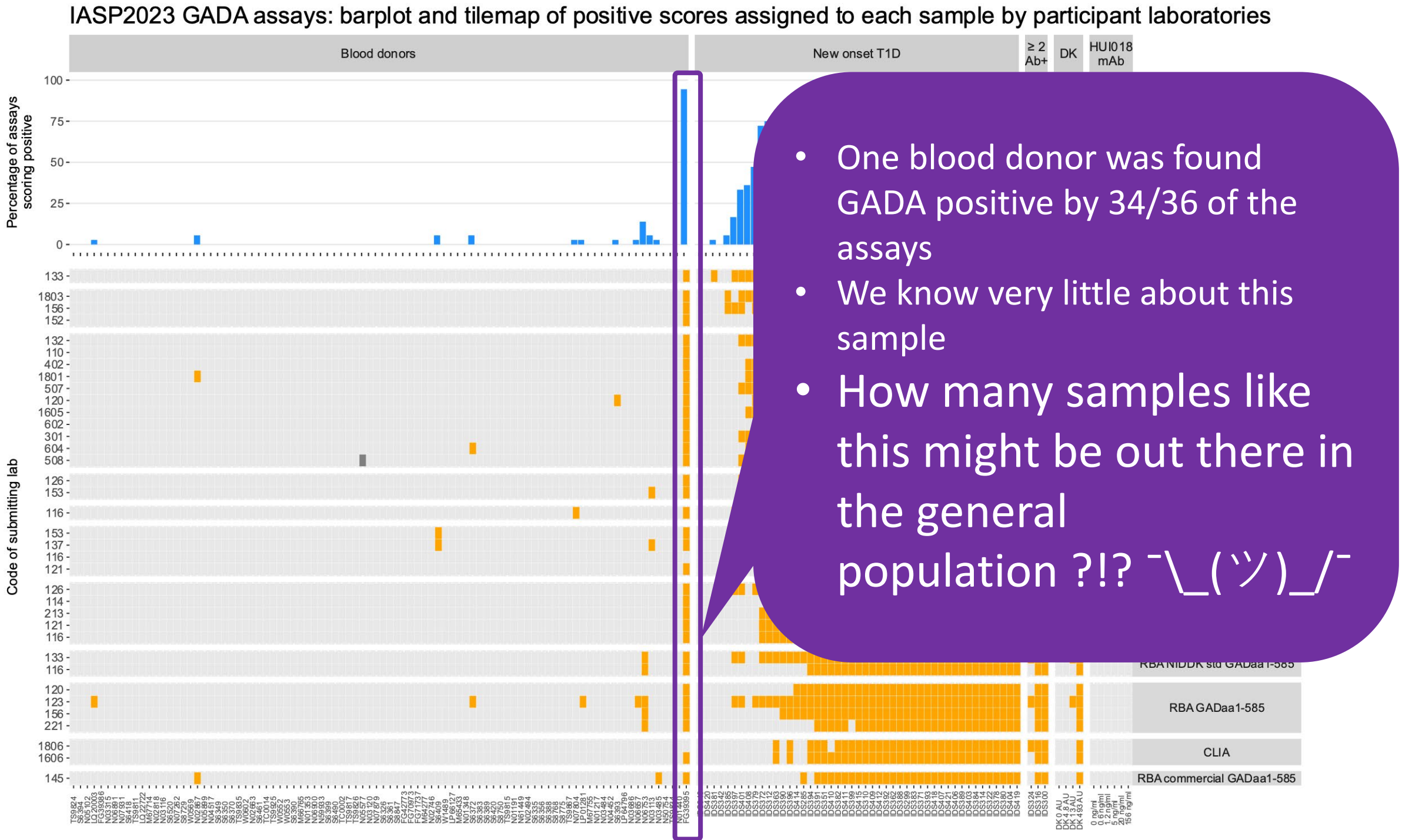
IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories



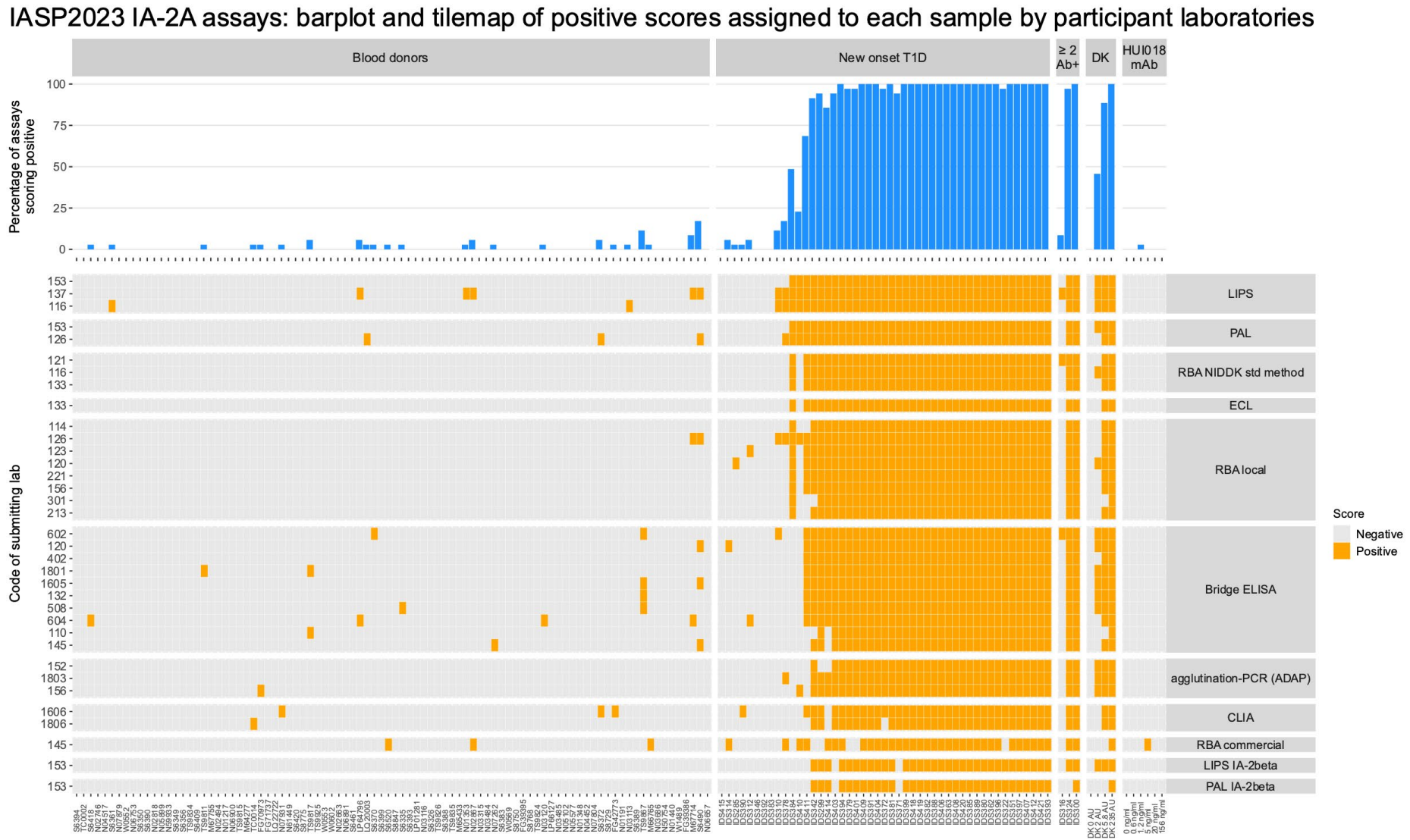
- Finally, the barplot shows the percentage of assays calling each sample GADA positive

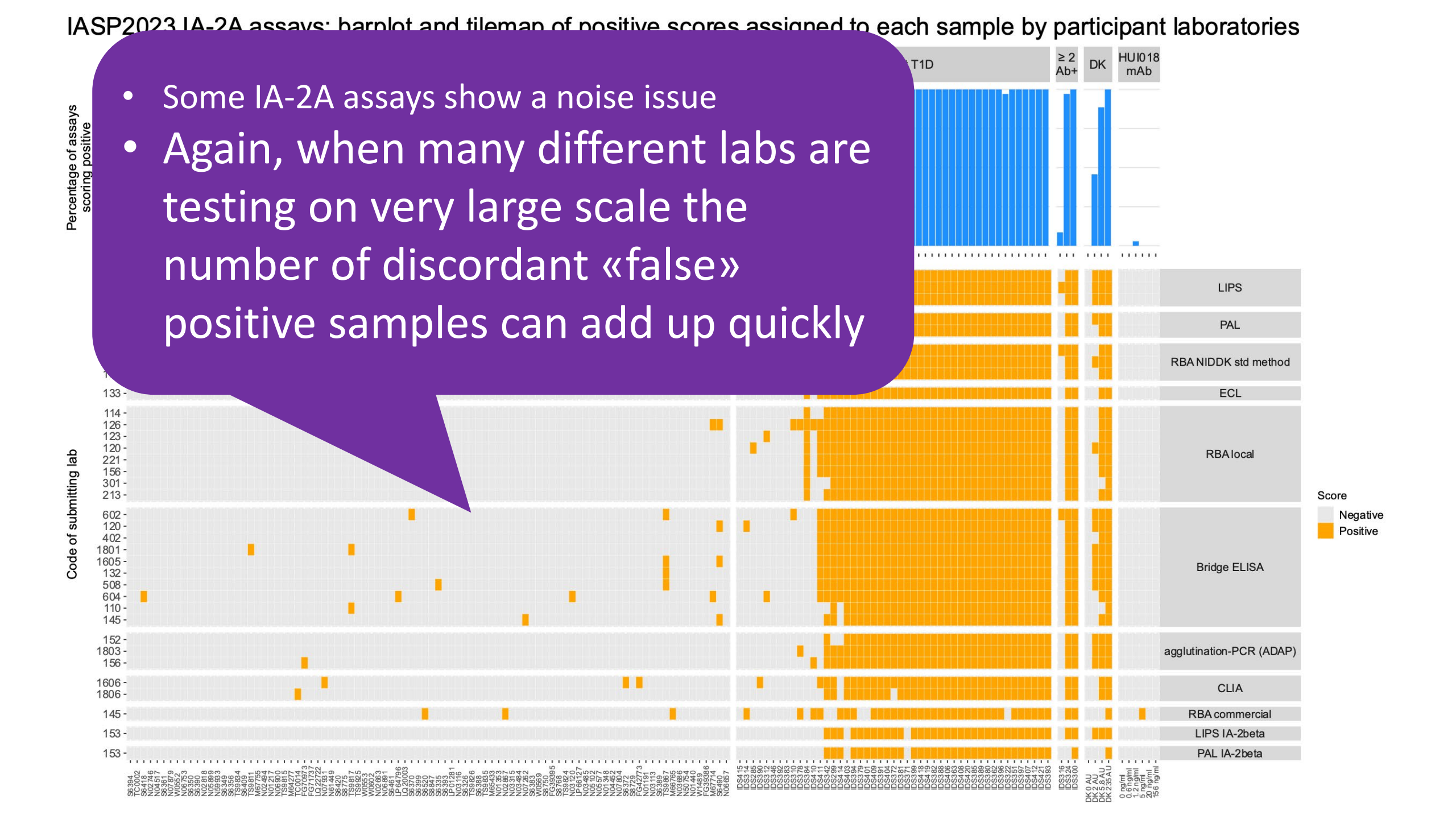

IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories
Blood donors
New onset T1D
≥ 2 Ab+
DK
HUI018 mAb
Percentage of assays scoring positive
100
75
50
25
0
Code of submitting lab
133
1803
156
152
ECL
agglutination-PCR (ADAP)
132
110
402
1801
507
120
1605
602
301
604
508
Bridge ELISA
126
153
PAL GADaa96-585
116
LIPS GADaa1-585
153
137
116
121
LIPS GADaa96-585
126
114
213
121
116
RBA GADaa96-585
133
116
RBA NIDDK std GADaa1-585
120
123
156
221
RBA GADaa1-585
1806
1606
CLIA
145
RBA commercial GADaa1-585
Score
Negative
Positive
DK 0 AU
DK 4.3 AU
DK 14 AU
DK 483 AU
0 ng/ml
0.6 ng/ml
1.2 ng/ml
5 ng/ml
20 ng/ml
156 ng/ml


IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories
Blood donors
New onset T1D
≥ 2 Ab+
DK
HUI018 mAb
Percentage of assays scoring positive
Code of submitting lab
ECL
agglutination-PCR (ADAP)
Bridge ELISA
PAL GADaa96-585
LIPS GADaa1-585
LIPS GADaa96-585
RBA GADaa96-585
RBA NIDDK std GADaa1-585
RBA GADaa1-585
CLIA
RBA commercial GADaa1-585
Score
Negative
Positive
Overall, high degree of concordance
Discordant T1D samples have relatively lower Ab levels
But on a very large scale the absolute number of discordant samples won't be neglible


IASP2023 GADA assays: barplot and tilemap of positive scores assigned to each sample by participant laboratories
Blood donors
New onset T1D
≥ 2 Ab+
DK
HUI018 mAb
Percentage of assays scoring positive
Code of submitting lab
RBA NIDDK std GADaa1-585
RBA GADaa1-585
CLIA
RBA commercial GADaa1-585
• One blood donor was found GADA positive by 34/36 of the assays
• We know very little about this sample
• How many samples like this might be out there in the general population ?!? ¯_(ツ)_/¯

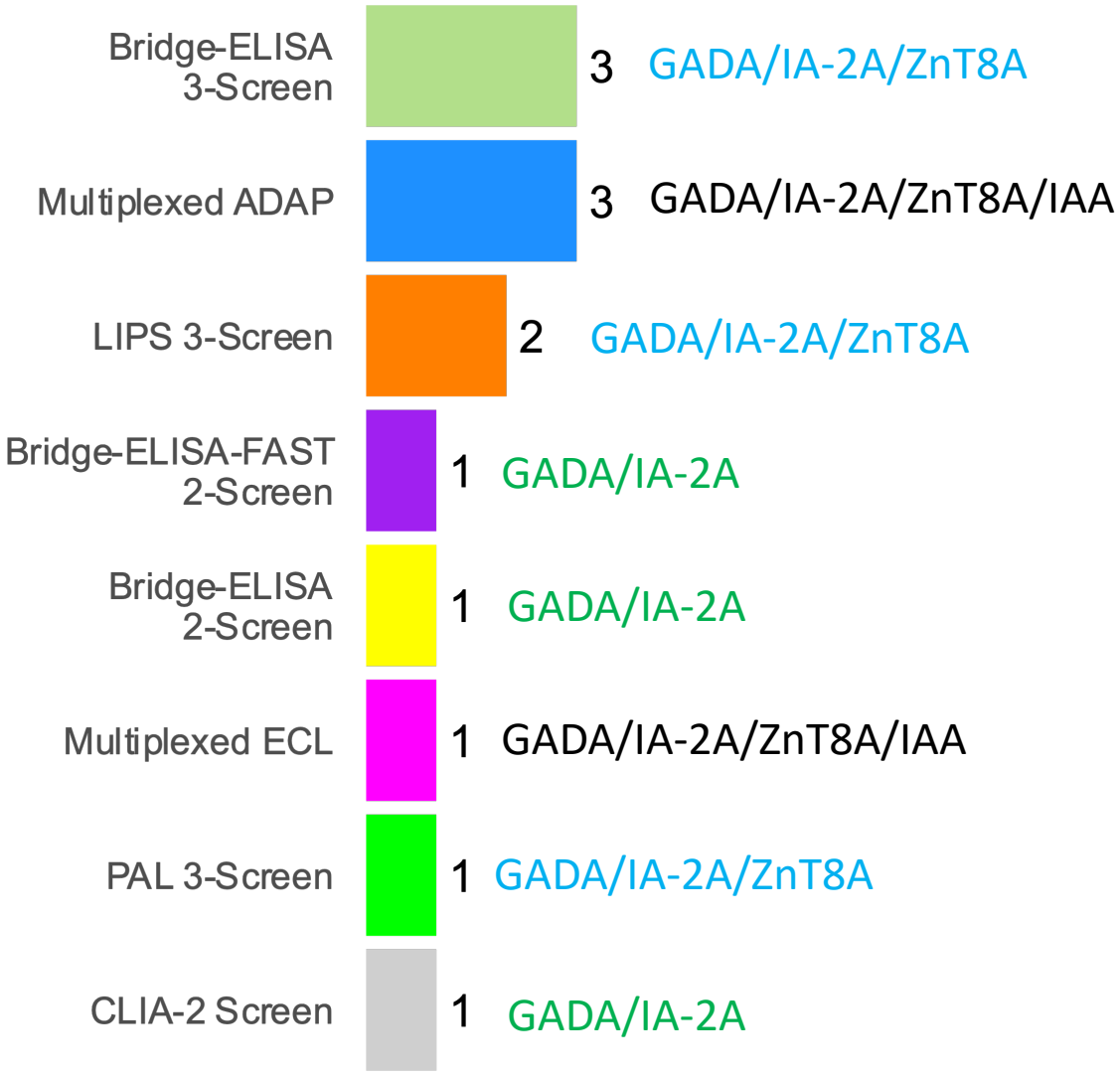
Are commercial kits “cleaner” and more reproducible? The workshop tells a more nuanced story: the IA-2A case

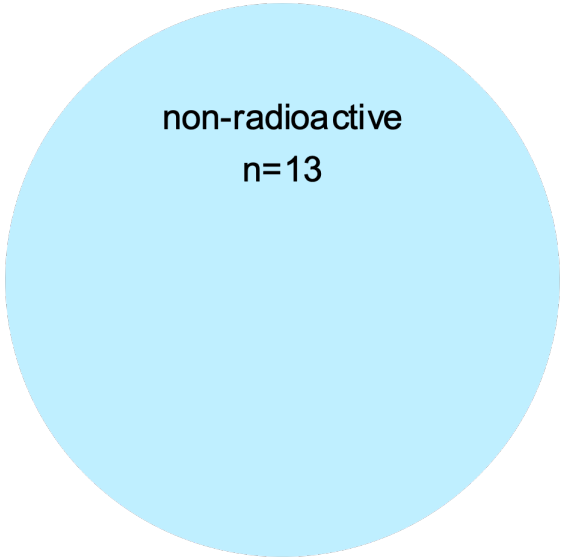


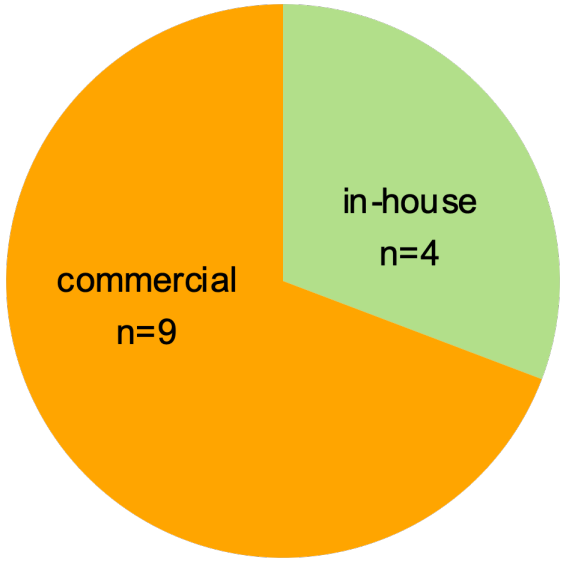


- Some IA-2A assays show a noise issue
- Again, when many different labs are testing on very large scale the number of discordant «false» positive samples can add up quickly

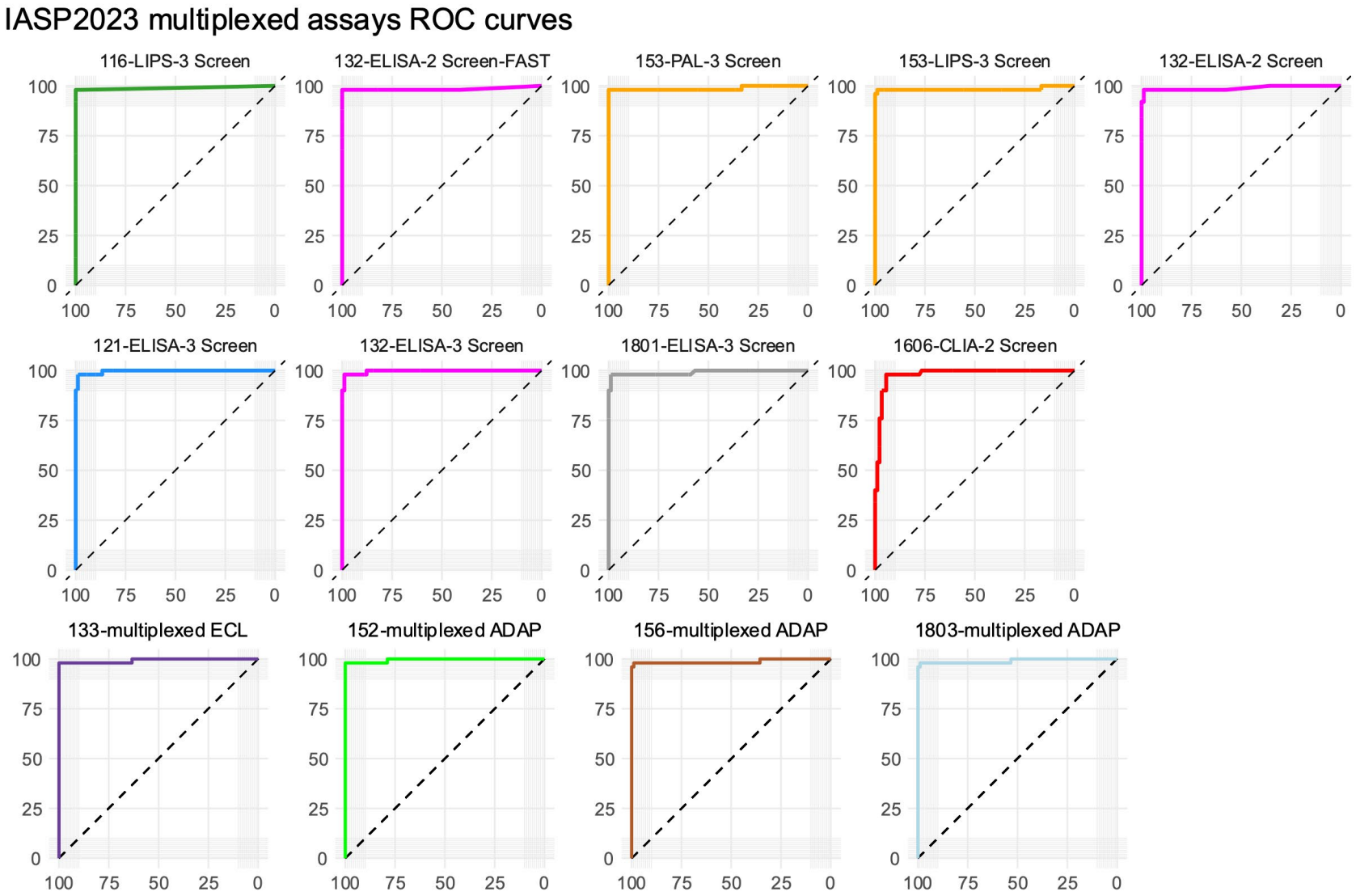
IASP perspective on multiplexed assays for screening



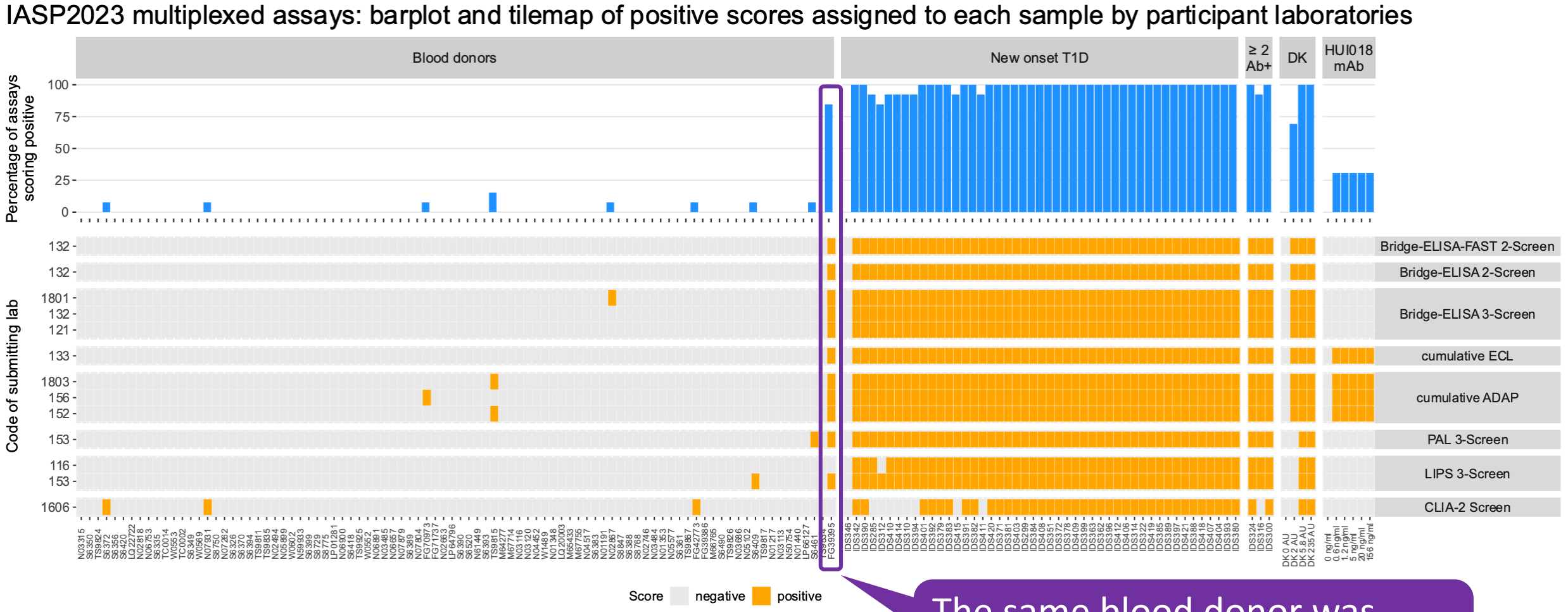




Several multiplexed assays achieved a very high performance in discriminating case from control in IASP2023



Multiplexed assays concordance was very high! But they were not totally spared from the issue we saw before for GADA



Another IASP perspective: assays might need thresholds adjustments for the new task of large-scale screening

Observation

- Thresholds are often based on relatively “small” training sample sets
- Commercial assays were developed by obviously very proficient lab for each specific format

Interpretation?

- A priori the chance to call false positives in the IASP T1D case cohort is low
- Labs/developers tend to adopt thresholds that identify also very weakly positive sera as Ab+ in the IASP case cohort
- The risk is that control samples with similar, low level of binding might be not so rare after all in the general population

Summary 1

- IASP is a successful program that is still helping the field of T1D autoantibody to identify good assays and assay formats
- Current non-radioisotopic multiplexed assays aimed at screening have shown great performance …… in IASP workshops
- Warning: only one of them (bridge-ELISA) has been applied in large-scale screening of capillary blood samples

Summary 2

- If the IASP core function of inter-assay comparison is expected to be extended to many more labs, then more resources (samples!) would be required
- IASP support the development and adoption of a new generation of standards for T1D autoantibodies and is considering strategies to help achieve that goal
- Changes/addition to the workshop format are under consideration to perform the assessment of limit of detection and reproducibility particularly for multiplexed assays, and to evaluate the impact of sample matrix on assay performance

Obviously, the previous timeline was a joke ……. we don't really care about what happened before antibody workshops!