

It's about time.

The Development of a Rapid Optimized Single Antigen
Bead (ROB) LABScreen® Protocol to Expedite HLA
Antibody Testing.

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Capital Health



Healthy People, Healthy Communities

LABScreen[®] single antigen bead (SAB) Luminex assay

- Uses the Lambda Array Beads Multi-analyte System.
- Number of advantages compared to FlowPRA SAB and ELISA.
 - ↑ number of analytes tested simultaneously
 - High throughput
 - Rapid analysis (Fusion software)
- Still, the procedure is time intensive and is not optimal for use in urgent cases....
 - Friday afternoons come to mind.....

LABScreen[®] single antigen bead (SAB) Luminex protocol

- Incubate beads (5 μ l) and serum 20 μ l (RT) 30 min.
- Wash x3 (5 min/spin) 15 min.
- Incubate with 100 μ l anti-IgG-PE, 1:100 dilution (RT) 30 min.
- Wash x2 (5min/spin) 10 min.
- **Total assay time** **1h 25 min.**

Evidence for
incubation time/reagent concentration?
wash times?

2h

Objectives

- To develop a rapid single antigen bead LABScreen protocol without compromising the sensitivity of the assay.
- Investigate the effects of:
 - Centrifugation time
 - Serum incubation time
 - Anti-IgG-PE incubation time
 - Anti-IgG-PE concentration



Effect of reduced spin time

- Standard

5 washes x 5 min = 25 min

1300 x g

- Rapid

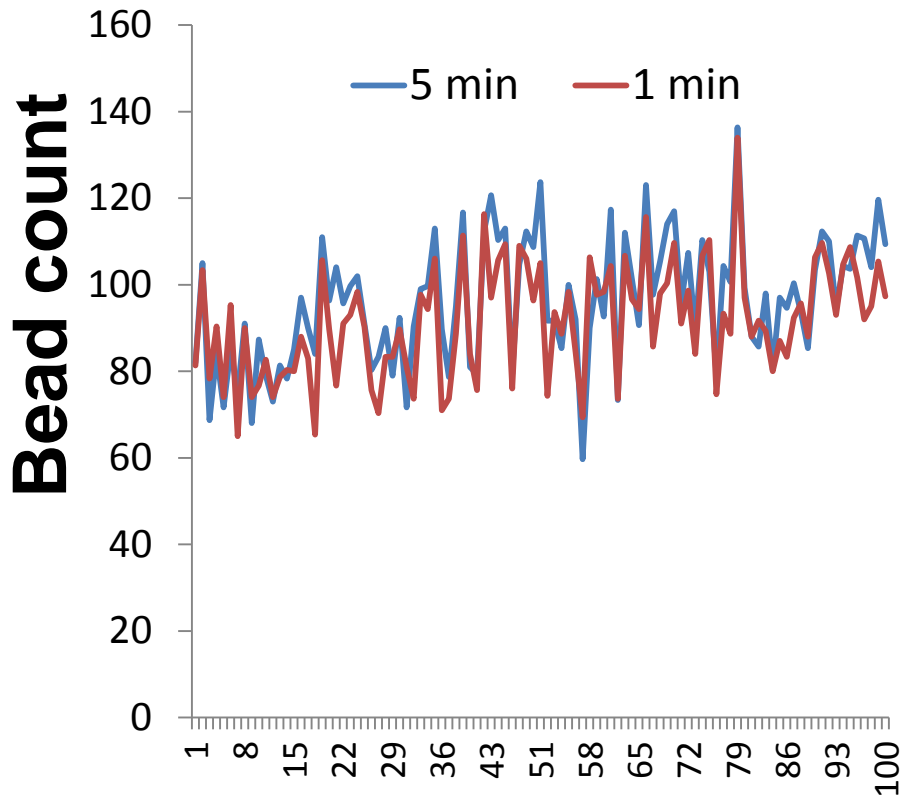
5 washes x 1 min = 5 min

1800 x g

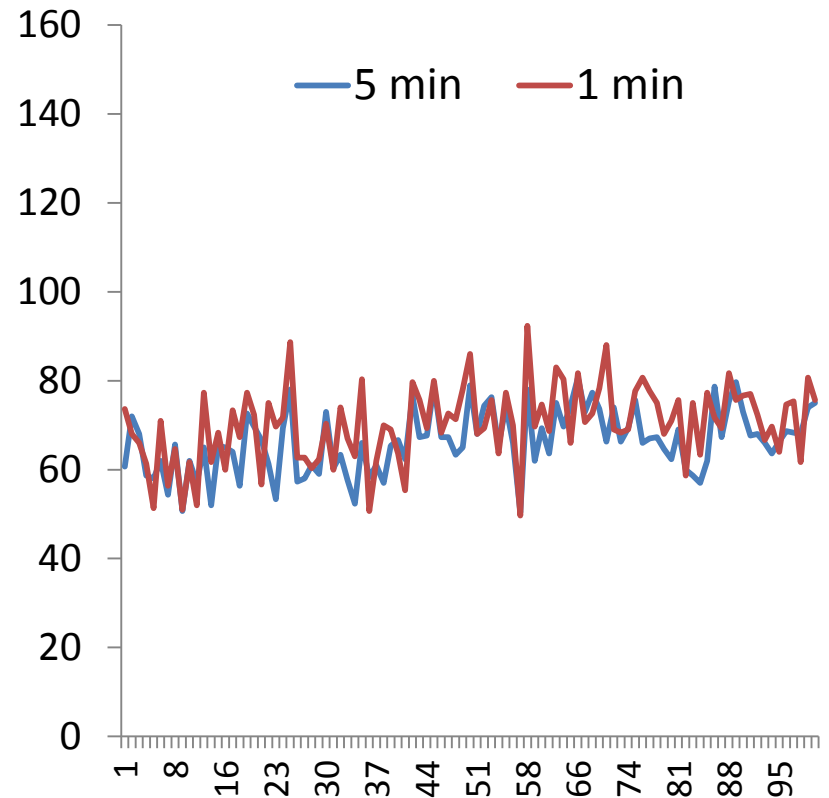


Effect of reduced spin time on bead counts

Class I beads



Class II beads



Bead number

Effect of reduced spin time

- Standard

5 washes x 5 min = 25 min

1300 x g

- Rapid

5 washes x 1 min = 5 min

1800 x g

No impact on bead counts or overall results
20 minutes saved!



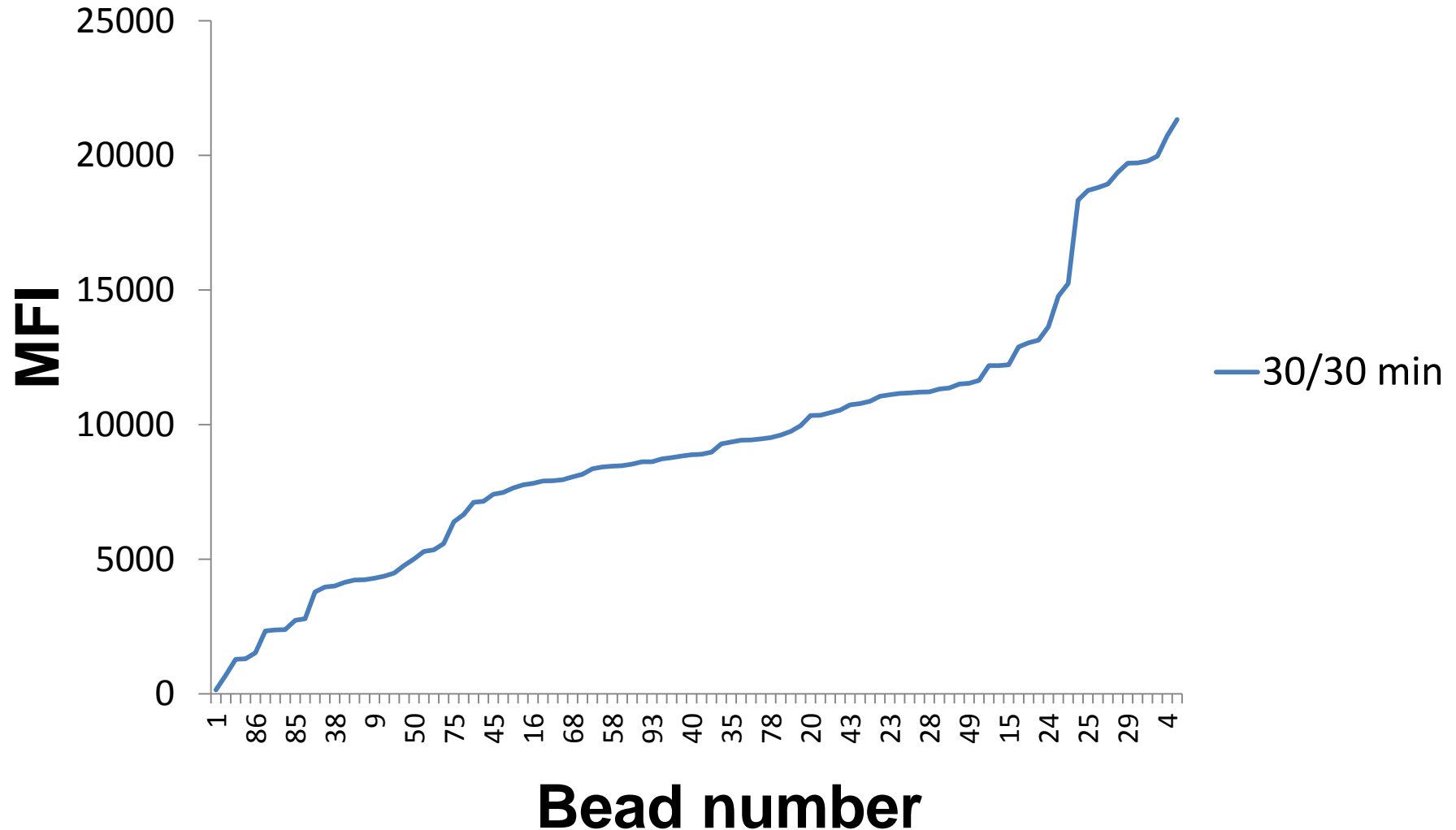
Effects of reduced incubation times

- Serum incubation time
- Anti-IgG-PE incubation time

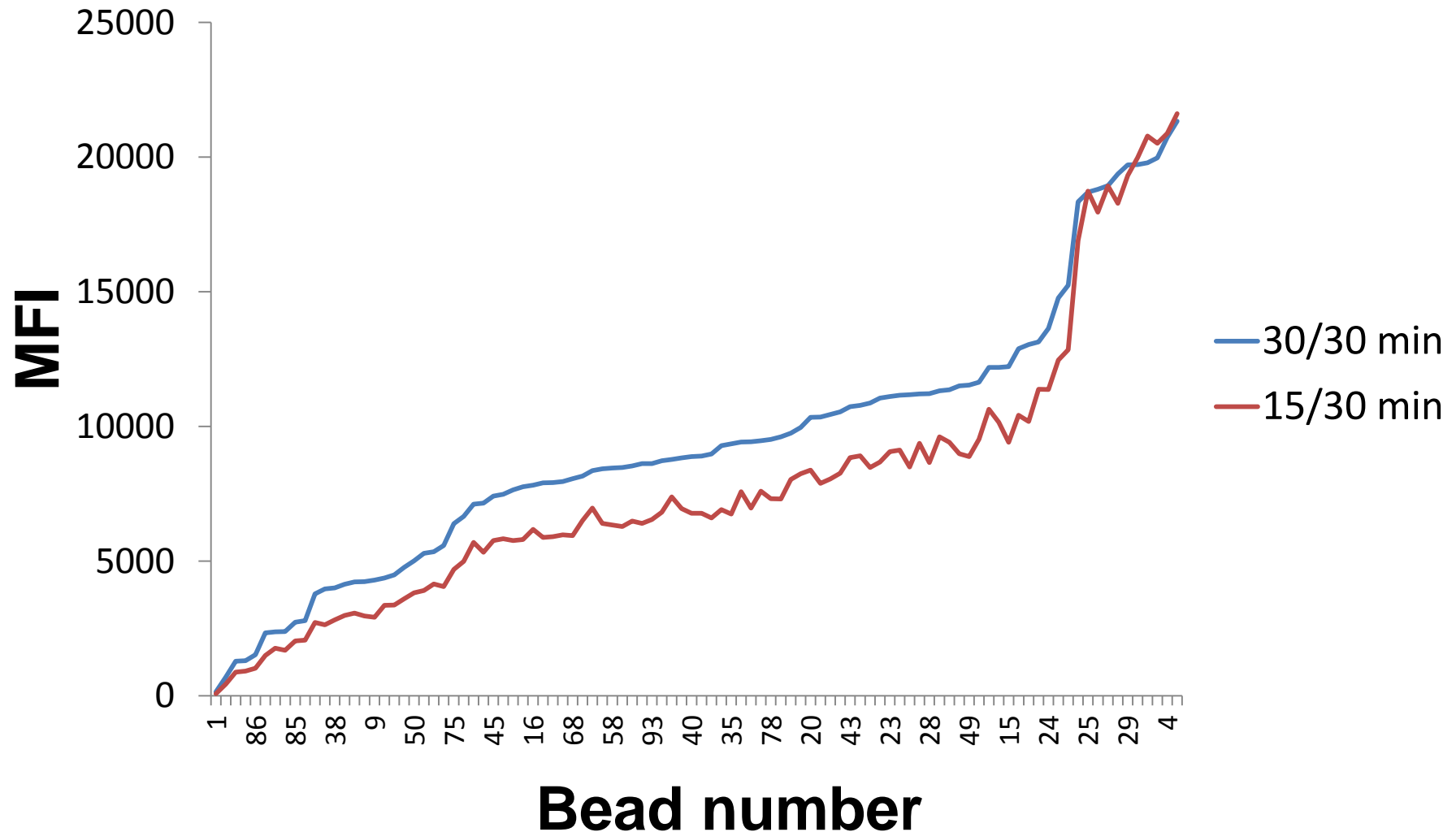


Effects of reduced incubation time

$\frac{1}{4}$ PPC, HLA class I

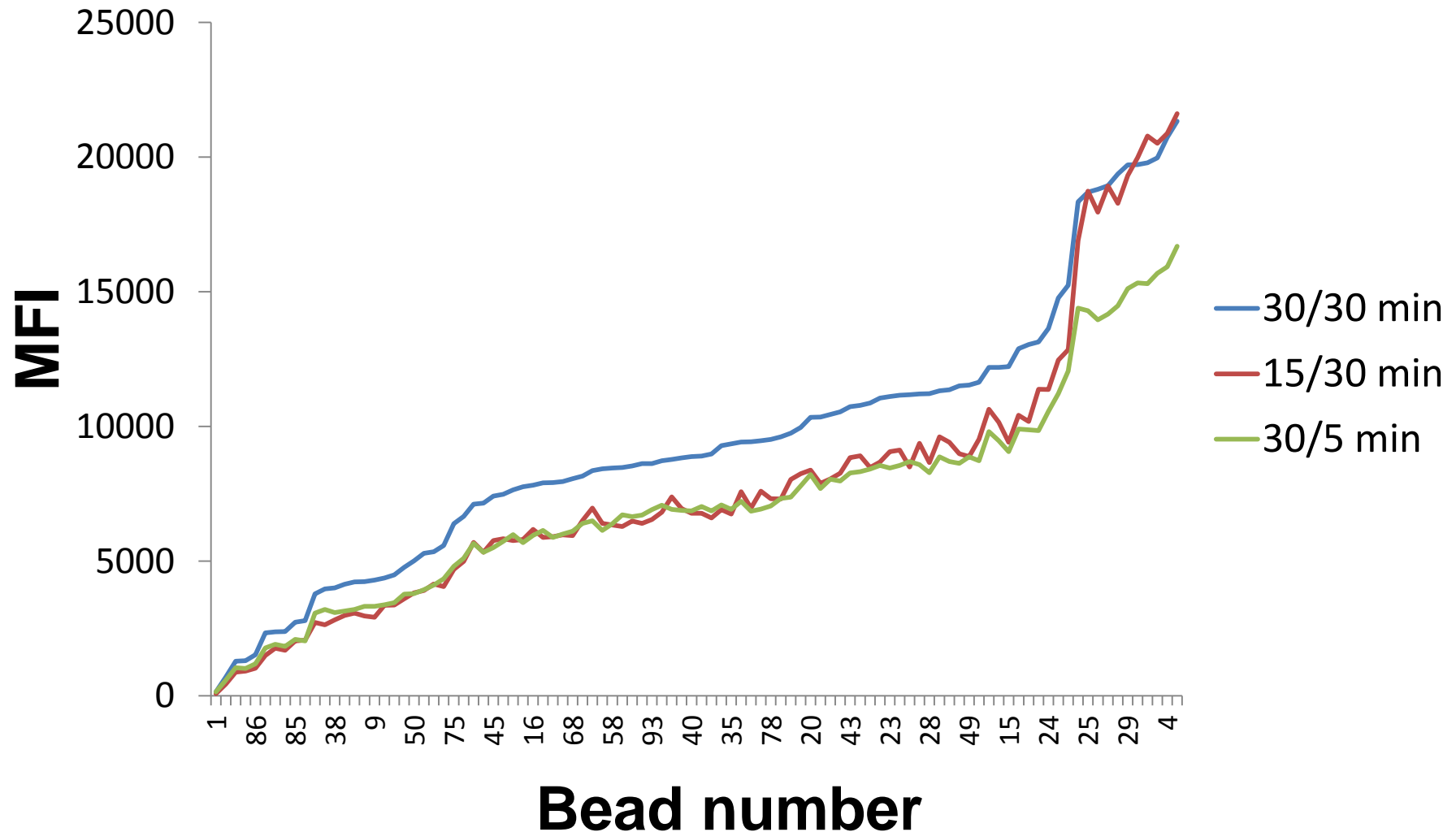


Effects of reduced incubation time $\frac{1}{4}$ PPC, HLA class I



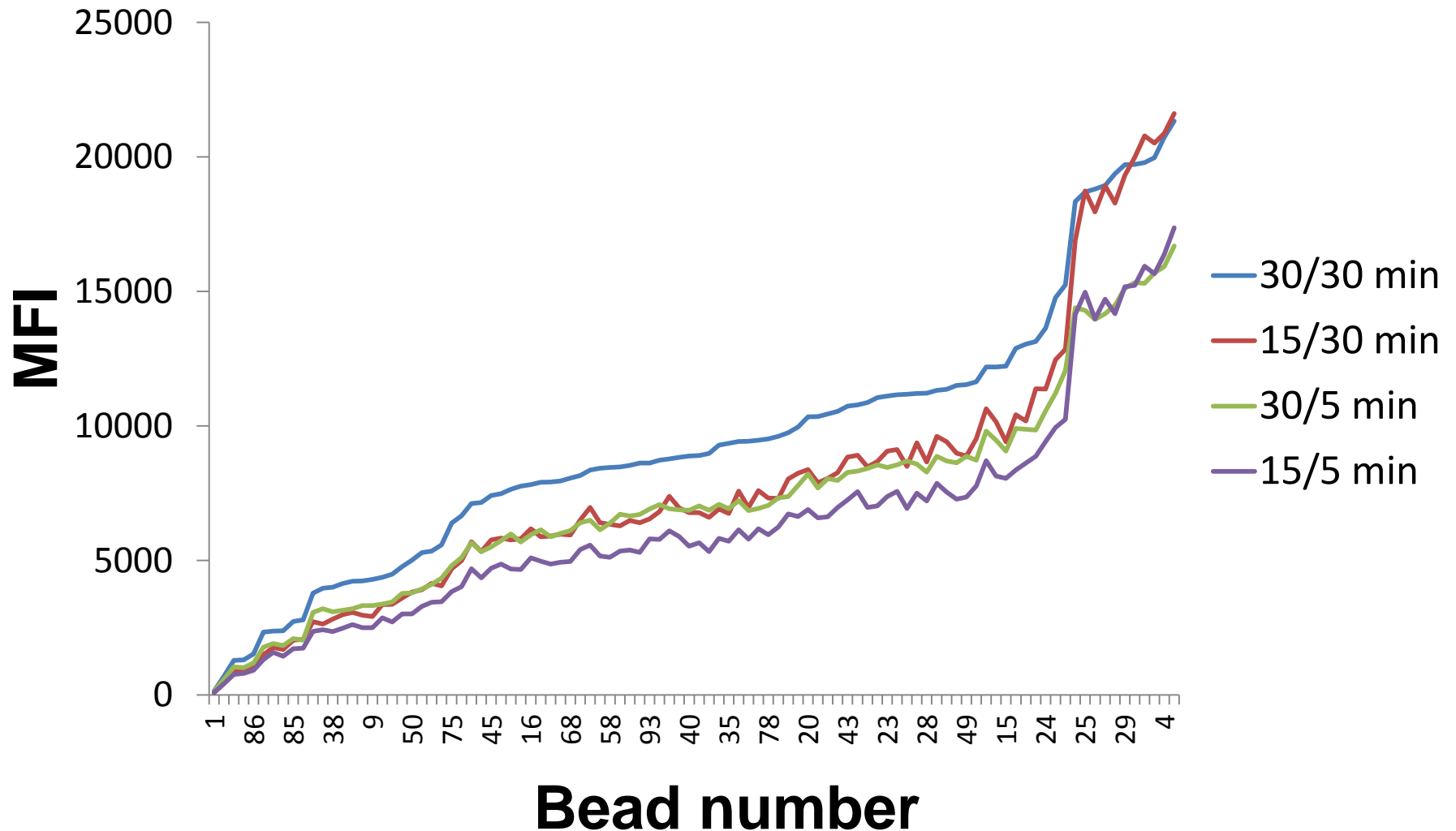
Effects of reduced incubation time

$\frac{1}{4}$ PPC, HLA class I

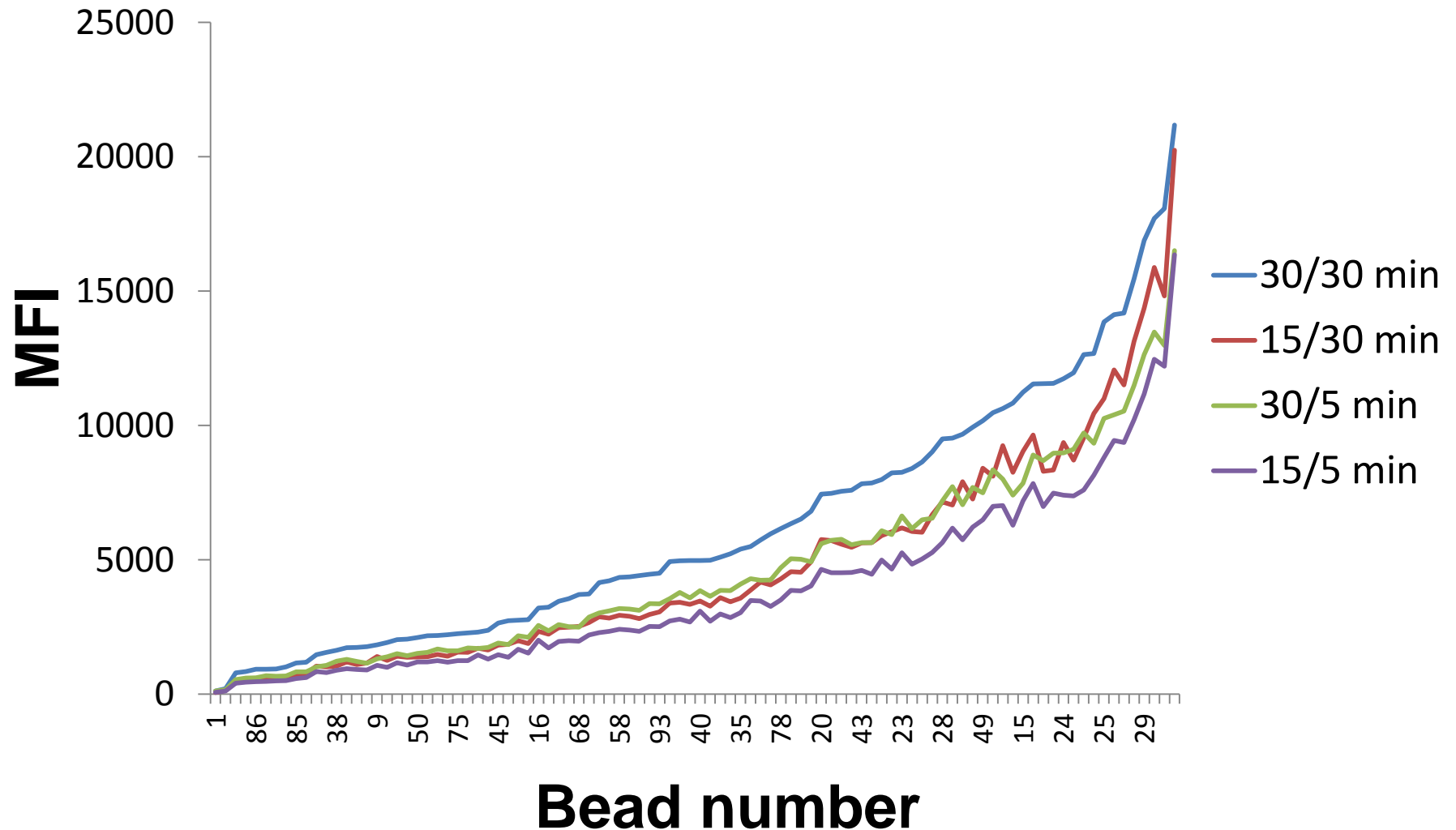


Effects of reduced incubation time

1/4 PPC, HLA class I



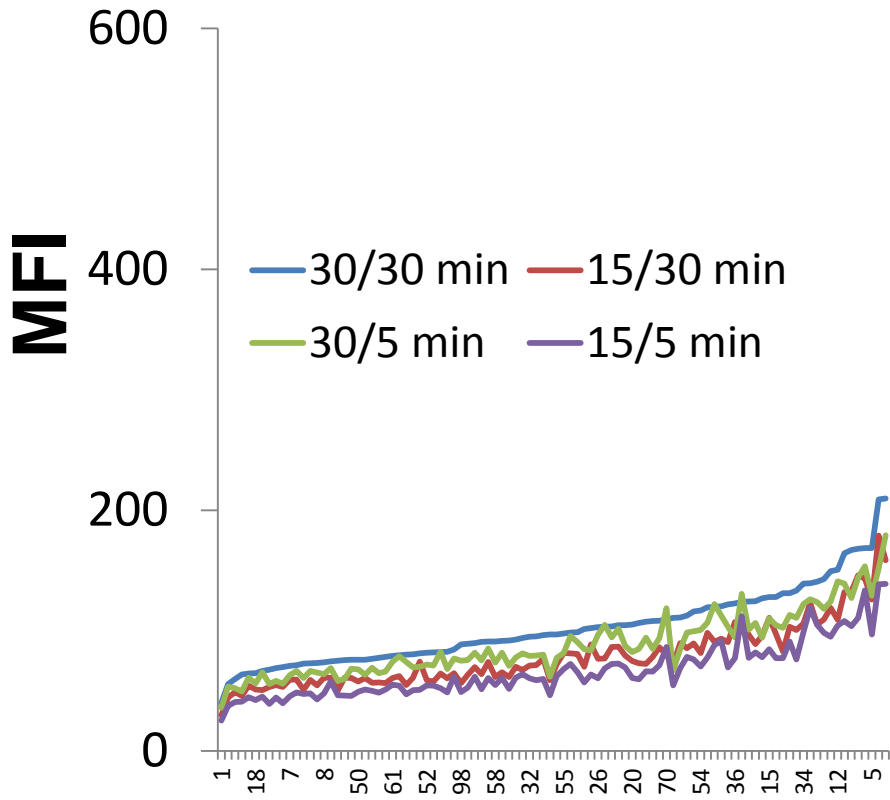
Effects of reduced incubation time $\frac{1}{4}$ PPC, HLA class II



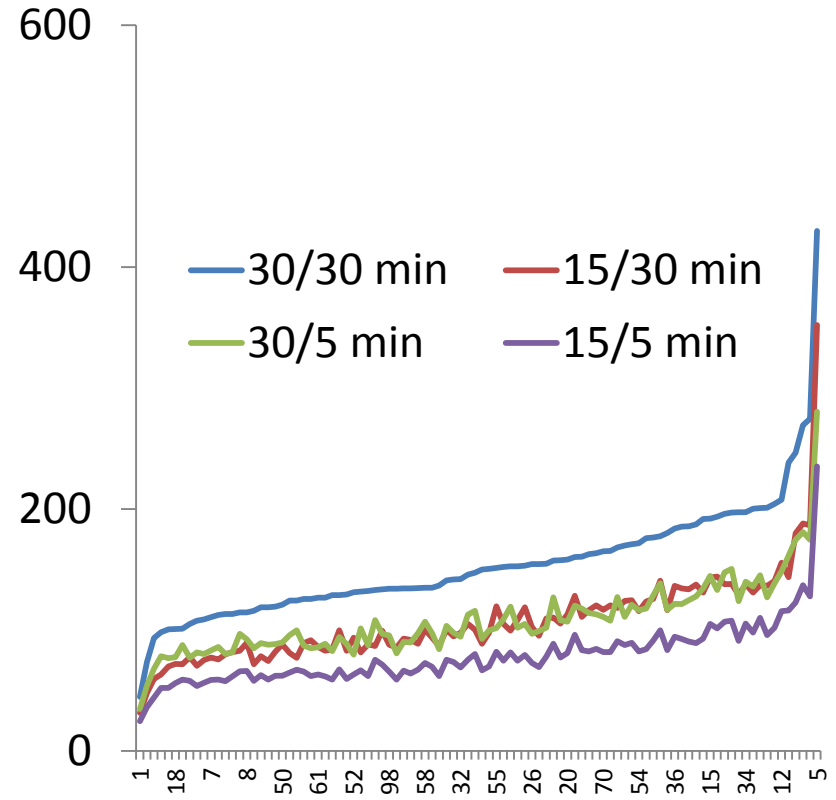
Effects of reduced incubation time

Negative control serum

Class I



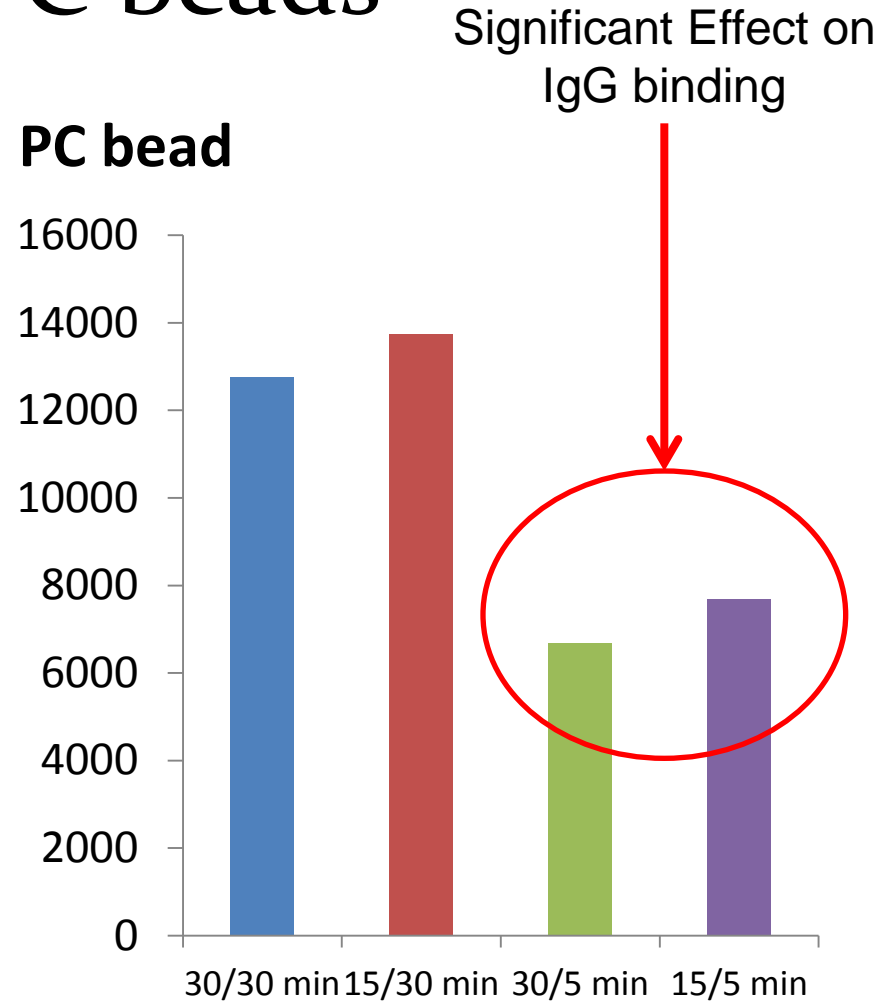
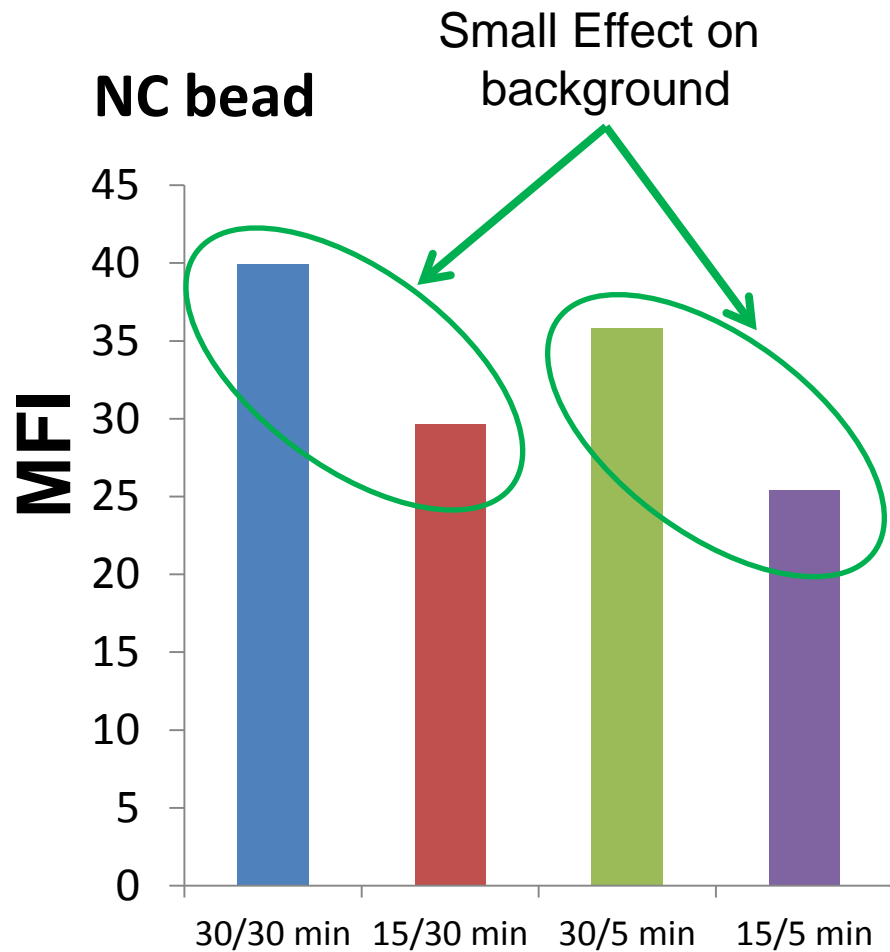
Class II



Bead number

Effects of reduced incubation time

NC and PC beads



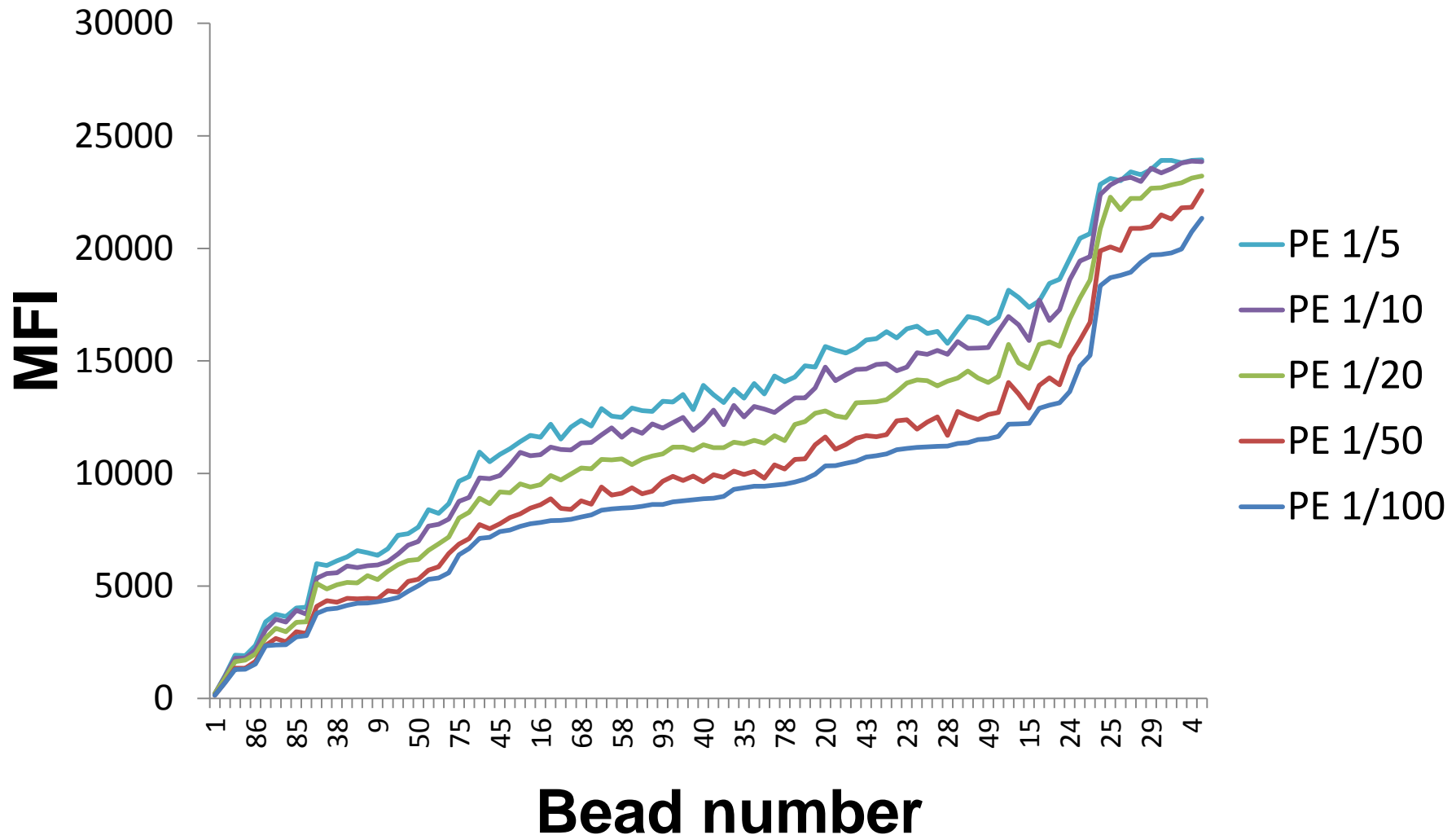
Serum/IgG-PE incubation time

Conclusion

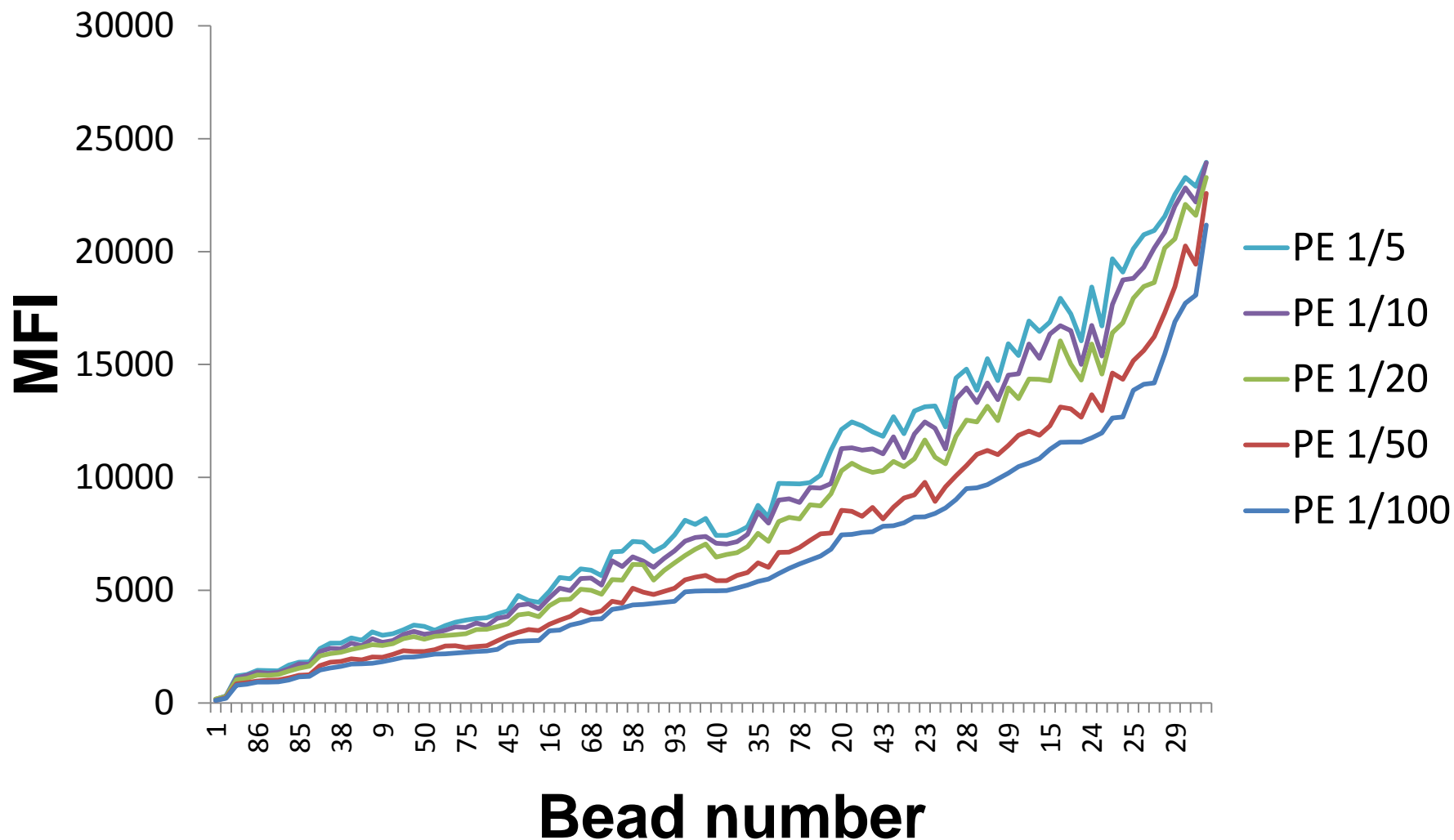
- Reduction in incubation time with serum and/or anti-IgG-PE results in decreased MFI values.
- Negligible impact on LSNC and NC bead reactivity.
- The degree of MFI decrease when incubation time with anti-IgG-PE was reduced was surprising.
- IgG-PE concentration appears to be sub-optimal?



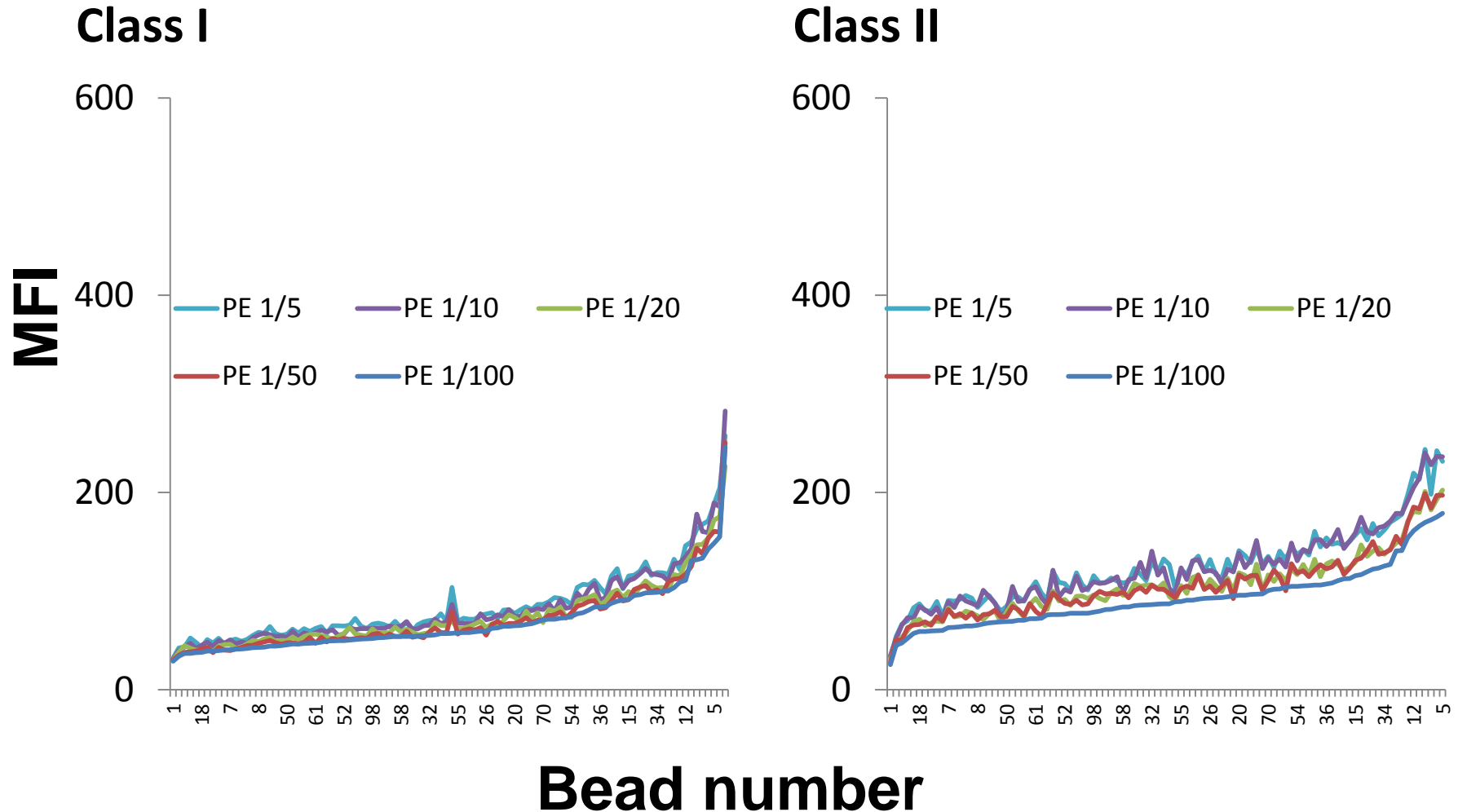
Effects of increasing IgG-PE concentration $\frac{1}{4}$ PPC, HLA class I



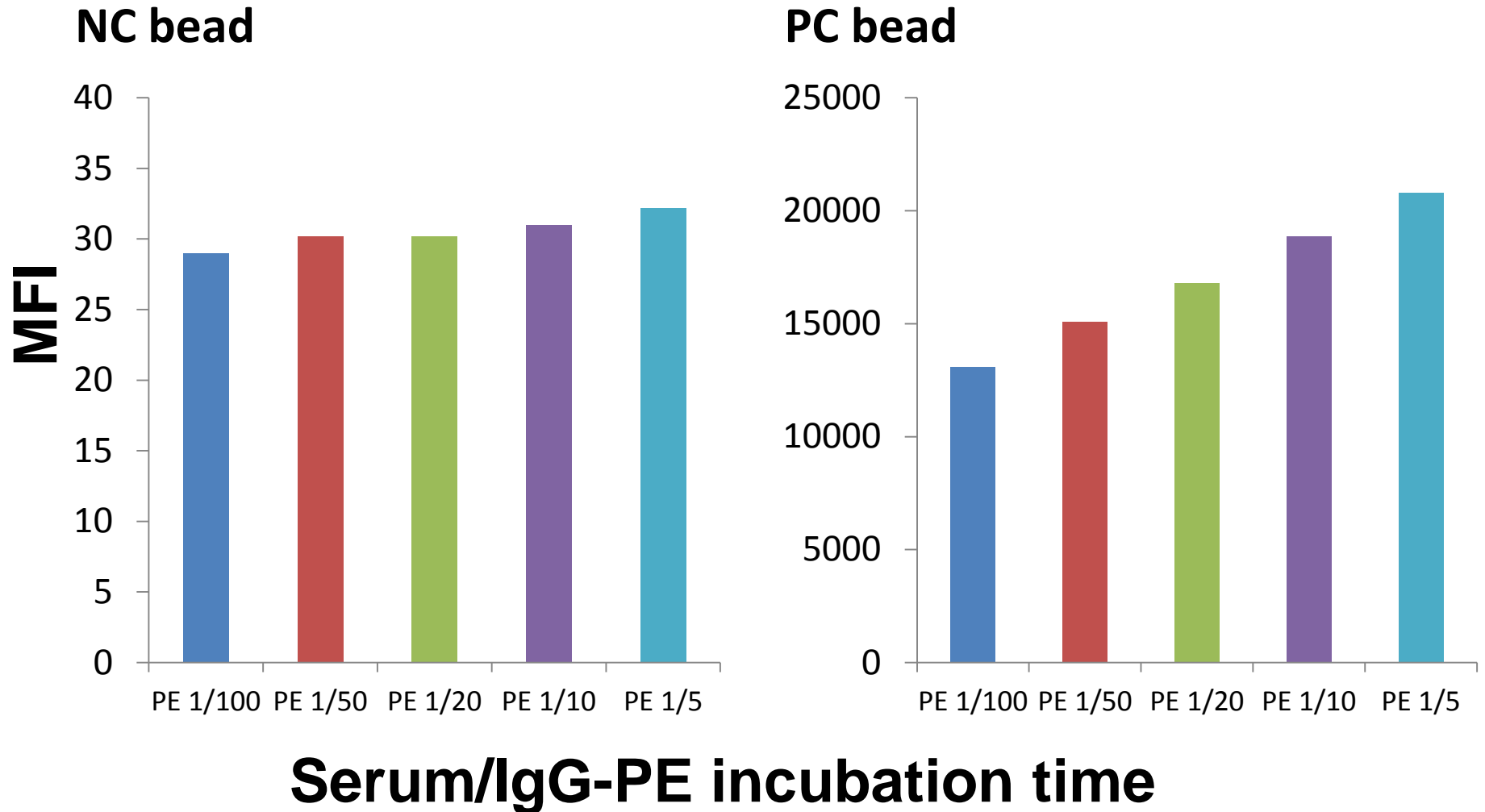
Effects of increasing IgG-PE concentration $\frac{1}{4}$ PPC, HLA class II



Effects of increasing IgG-PE concentration Negative Control Serum



Effects of increasing IgG-PE concentration NC and PC beads

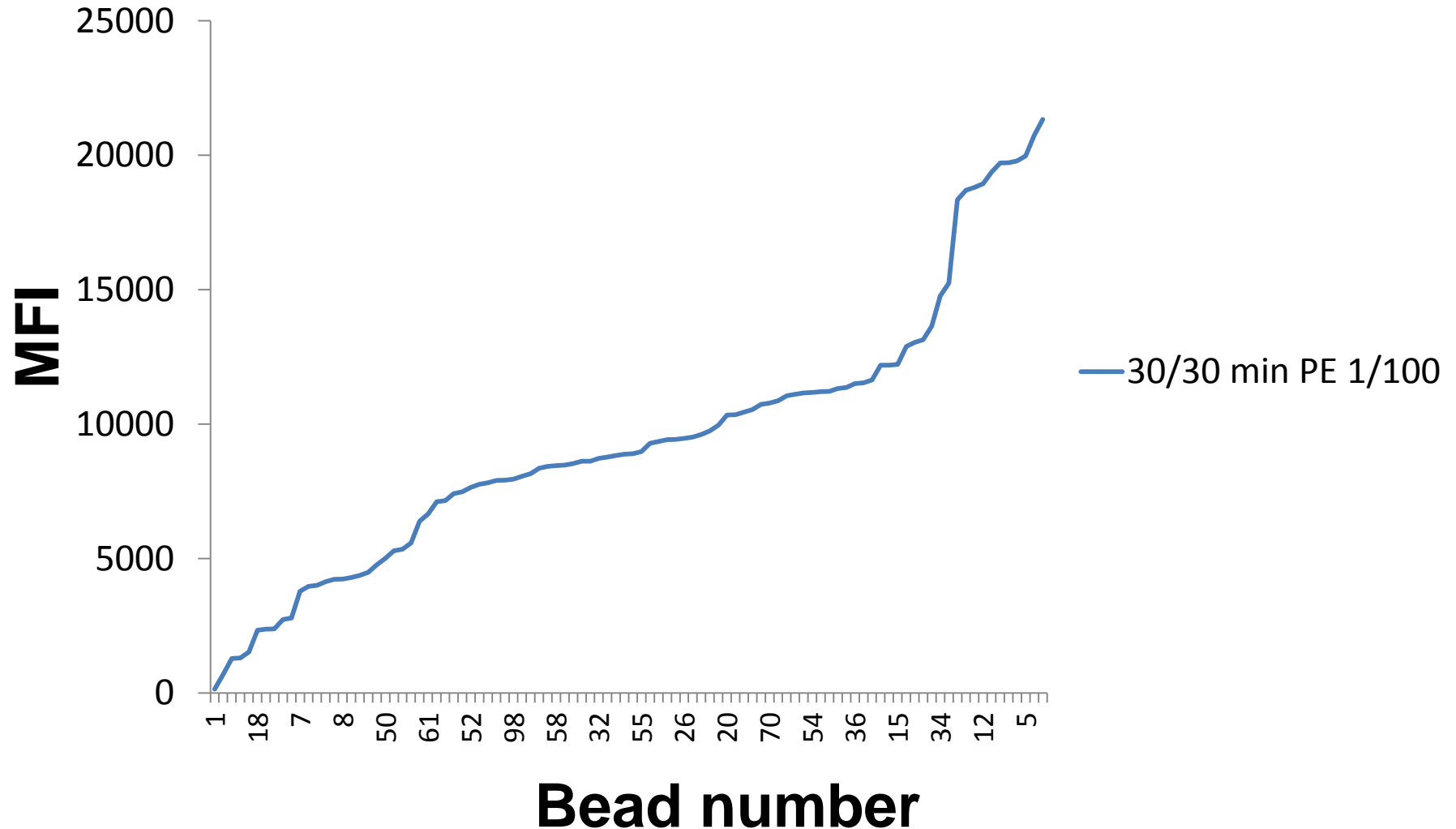


Anti-IgG-PE titration conclusion:

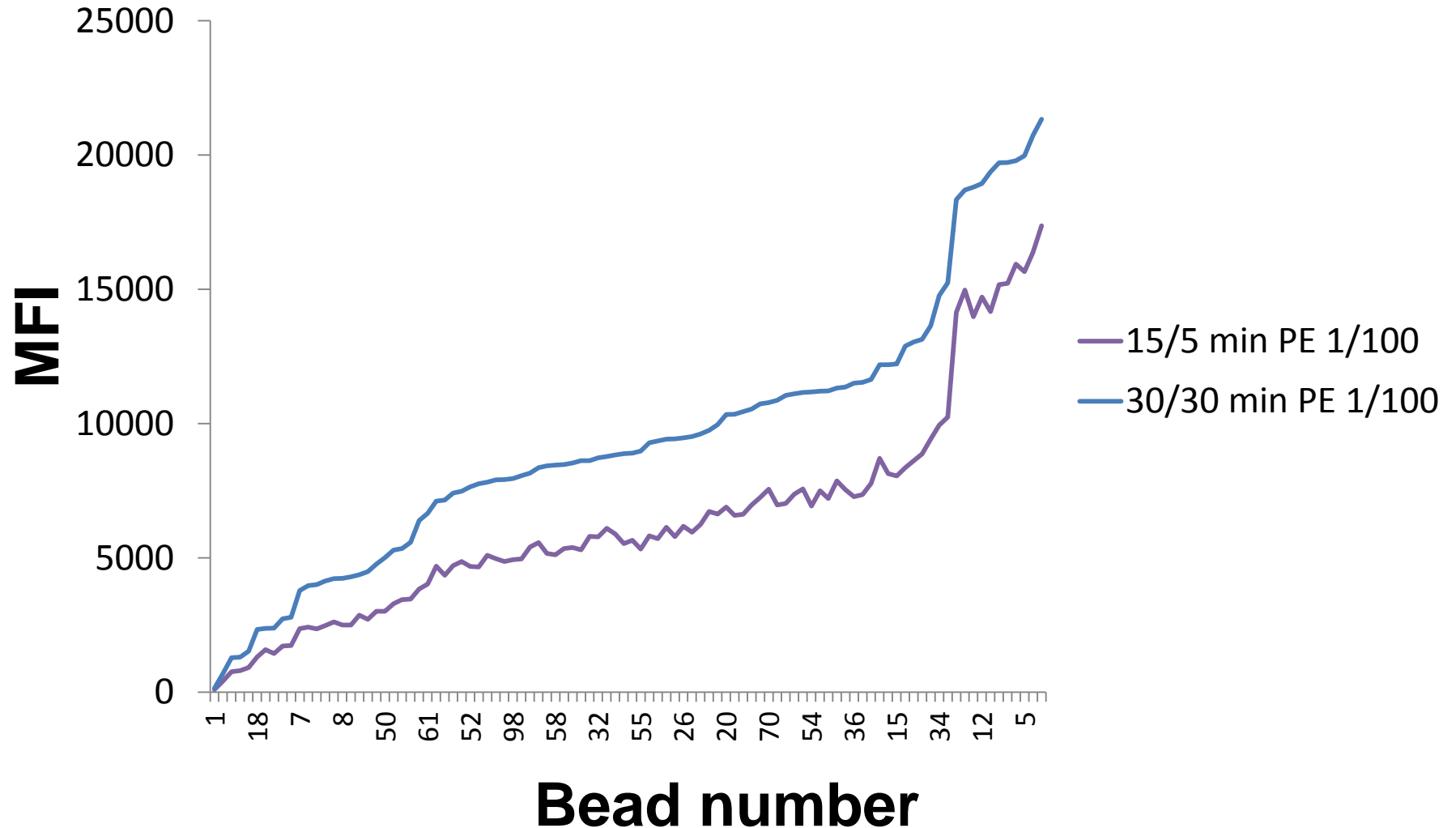
- Increasing the anti-IgG-PE concentration from 1:100 to 1:5 increases MFI in the standard assay including PC bead MFI.
- Negligible effect on background.
- Can we compensate for reduced MFI values in the 15/5 min protocol by optimizing the concentration of anti-IgG-PE?



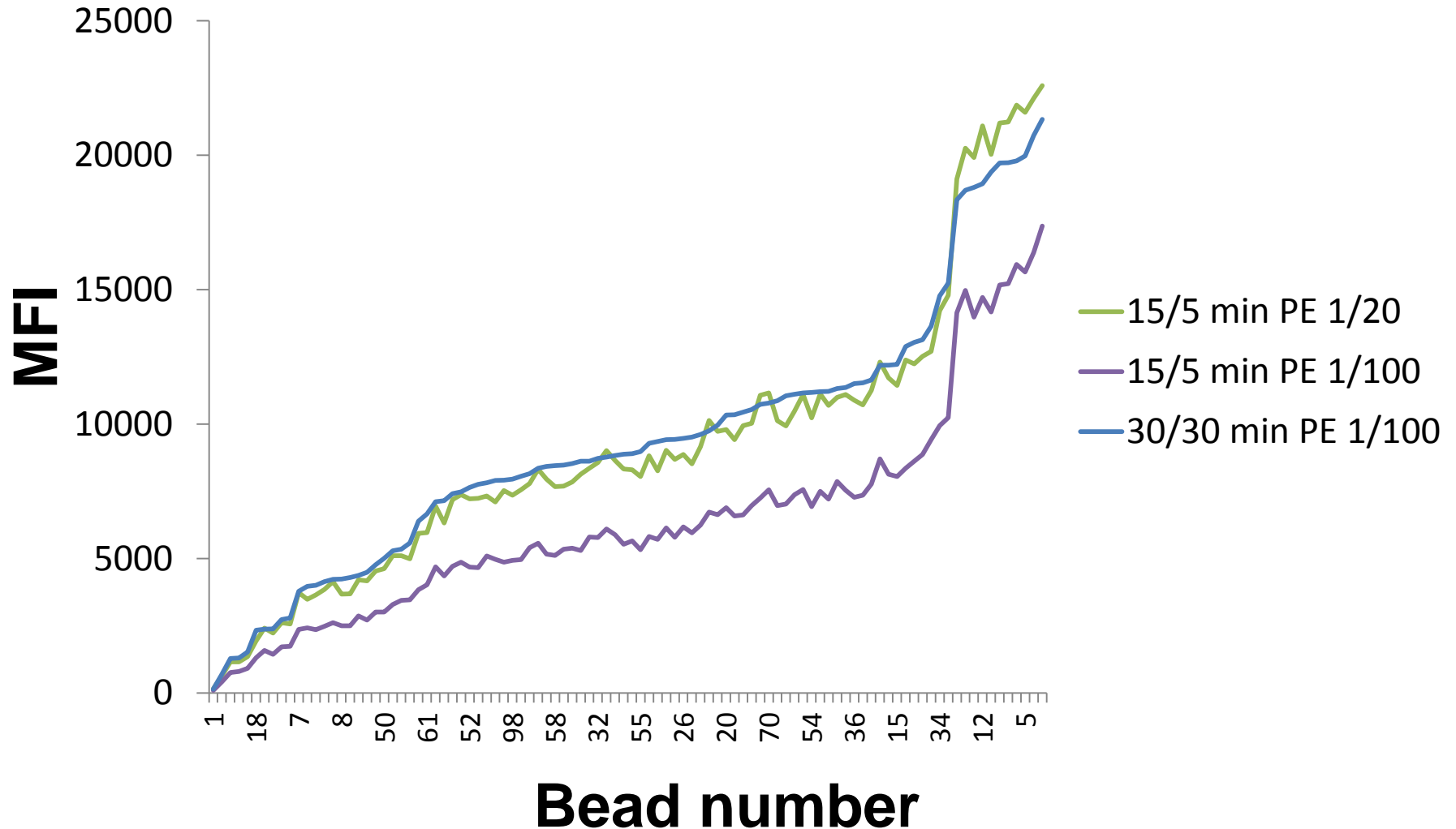
Effects of increasing IgG-PE concentration on MFI in 15/5 protocol PPC, HLA class I



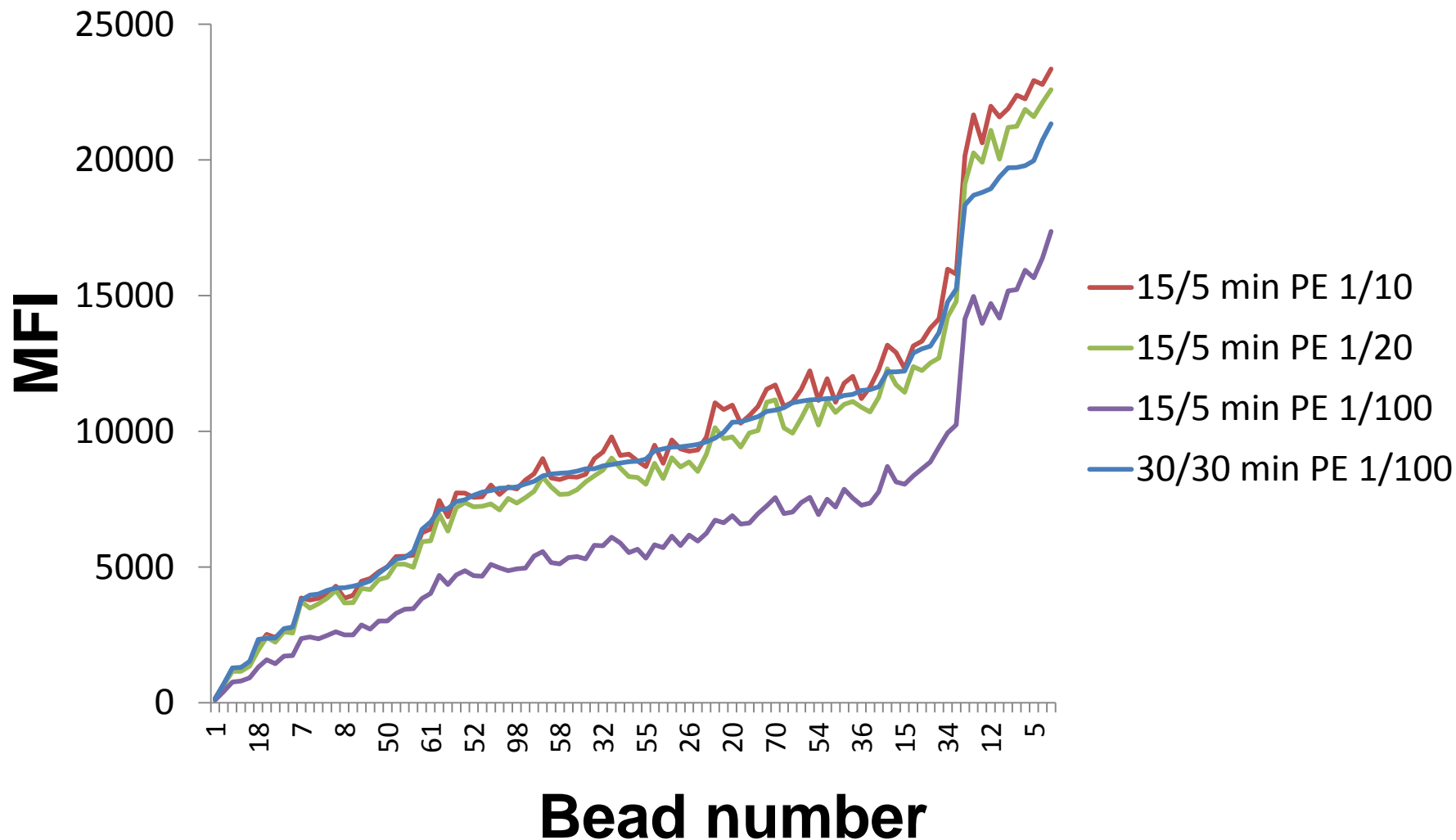
Effects of increasing IgG-PE concentration on MFI in 15/5 protocol PPC, HLA class I



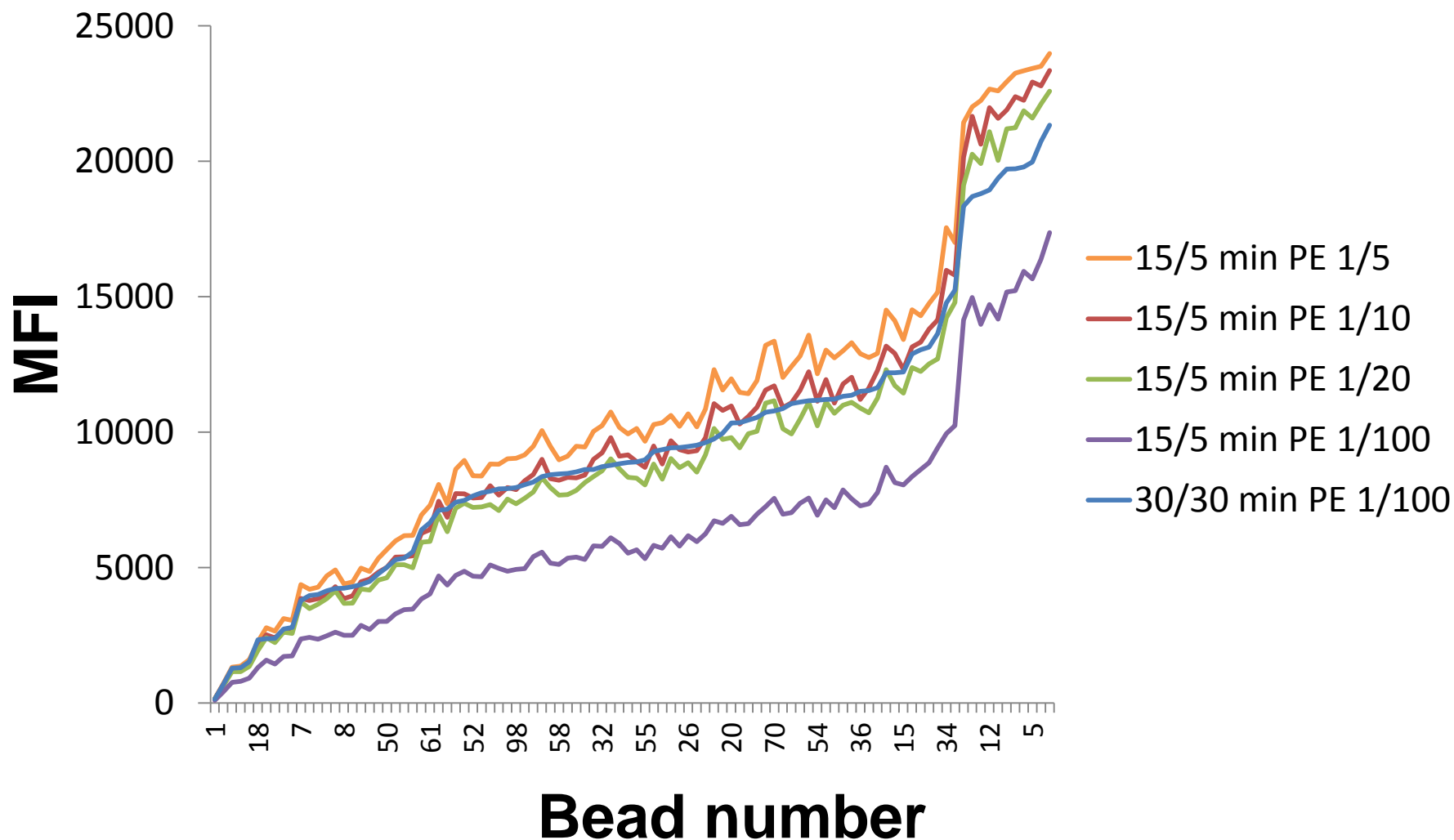
Effects of increasing IgG-PE concentration on MFI in 15/5 protocol PPC, HLA class I



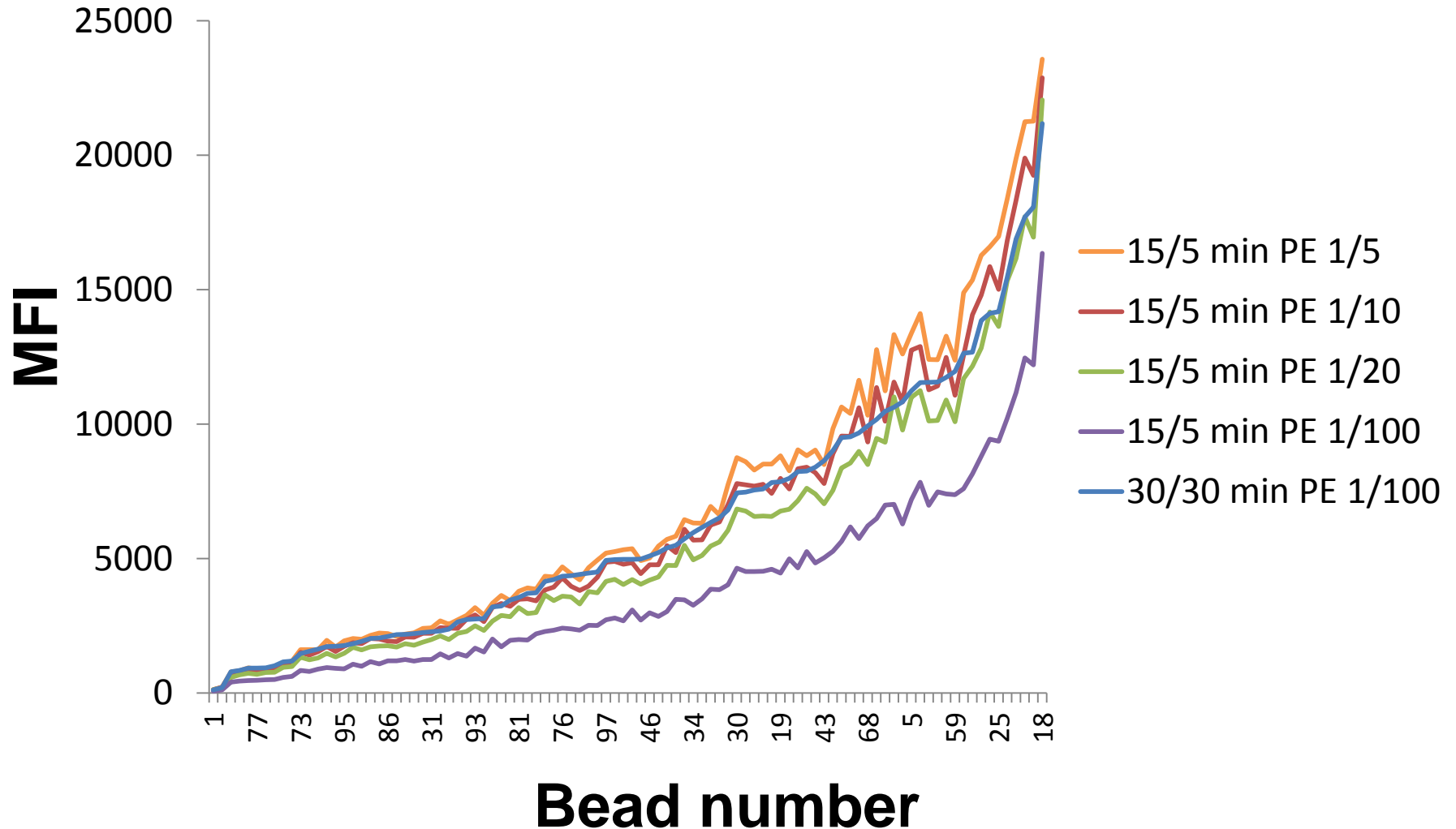
Effects of increasing IgG-PE concentration on MFI in 15/5 protocol PPC, HLA class I



Effects of increasing IgG-PE concentration on MFI in 15/5 protocol PPC, HLA class I



Effects of increasing IgG-PE concentration on MFI in 15/5 protocol PPC, HLA class II



Conclusion

- Increasing concentration of anti-IgG-PE compensates for the reduction in incubation times.
- IgG-PE concentration of 1:10 closely matches MFI obtained with the standard assay.



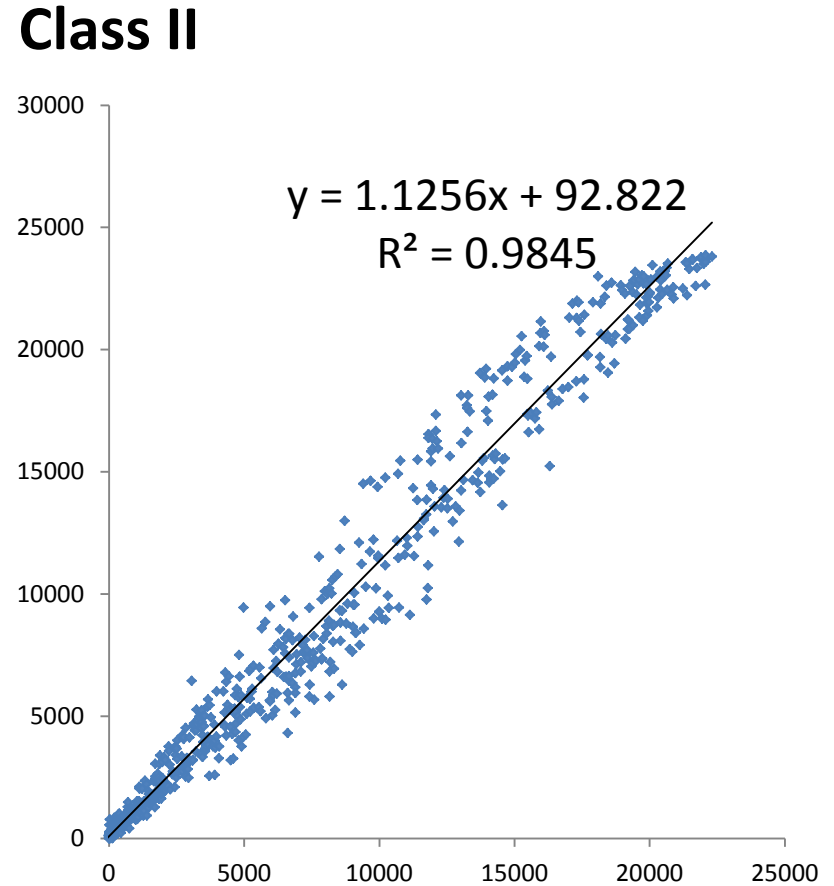
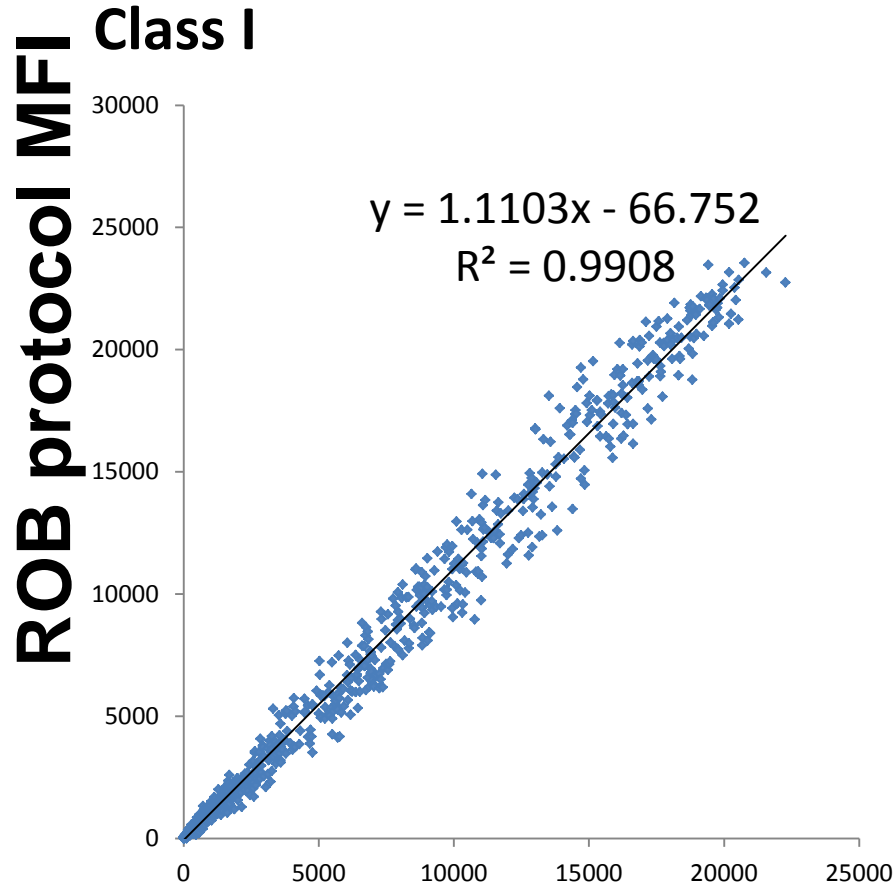
Rapid Optimized SAB (ROB) LABScreen[®] Protocol

- Incubate beads (2.5 μ l) and serum 25 μ l (RT) 15 min.
- Wash x3 (1 min/spin) 3 min.
- Incubate with 20 μ l anti-IgG-PE, 1:10 dilution (RT) 5 min.
- Wash x2 (5min/spin) 2 min.
- **Total assay time 25 min.**

70% time reduction!

Standard vs ROB protocol, MFI correlation

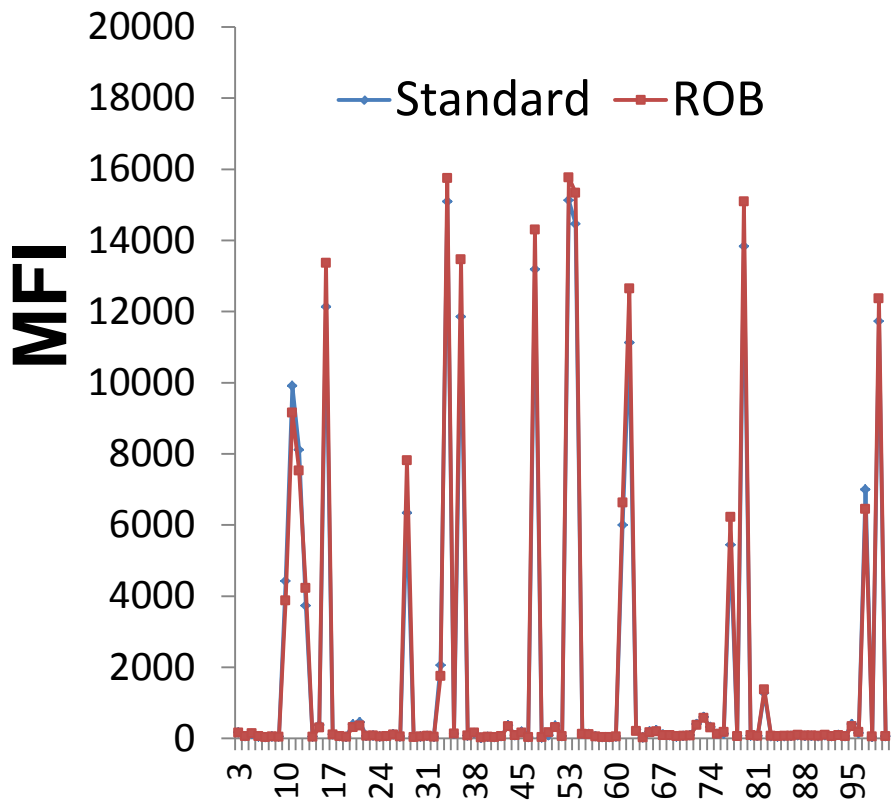
8 patient, 9 ASHI PT, 3 ABH PT sera



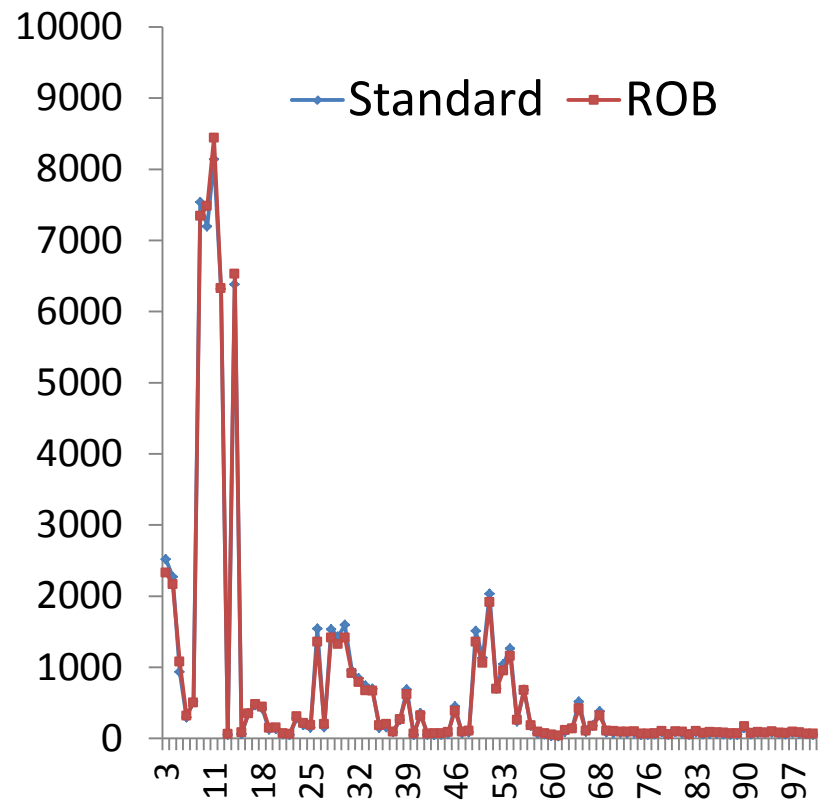
Standard Assay MFI

Representative Serum Reactivity Standard vs ROB protocol

AC-463 Class I

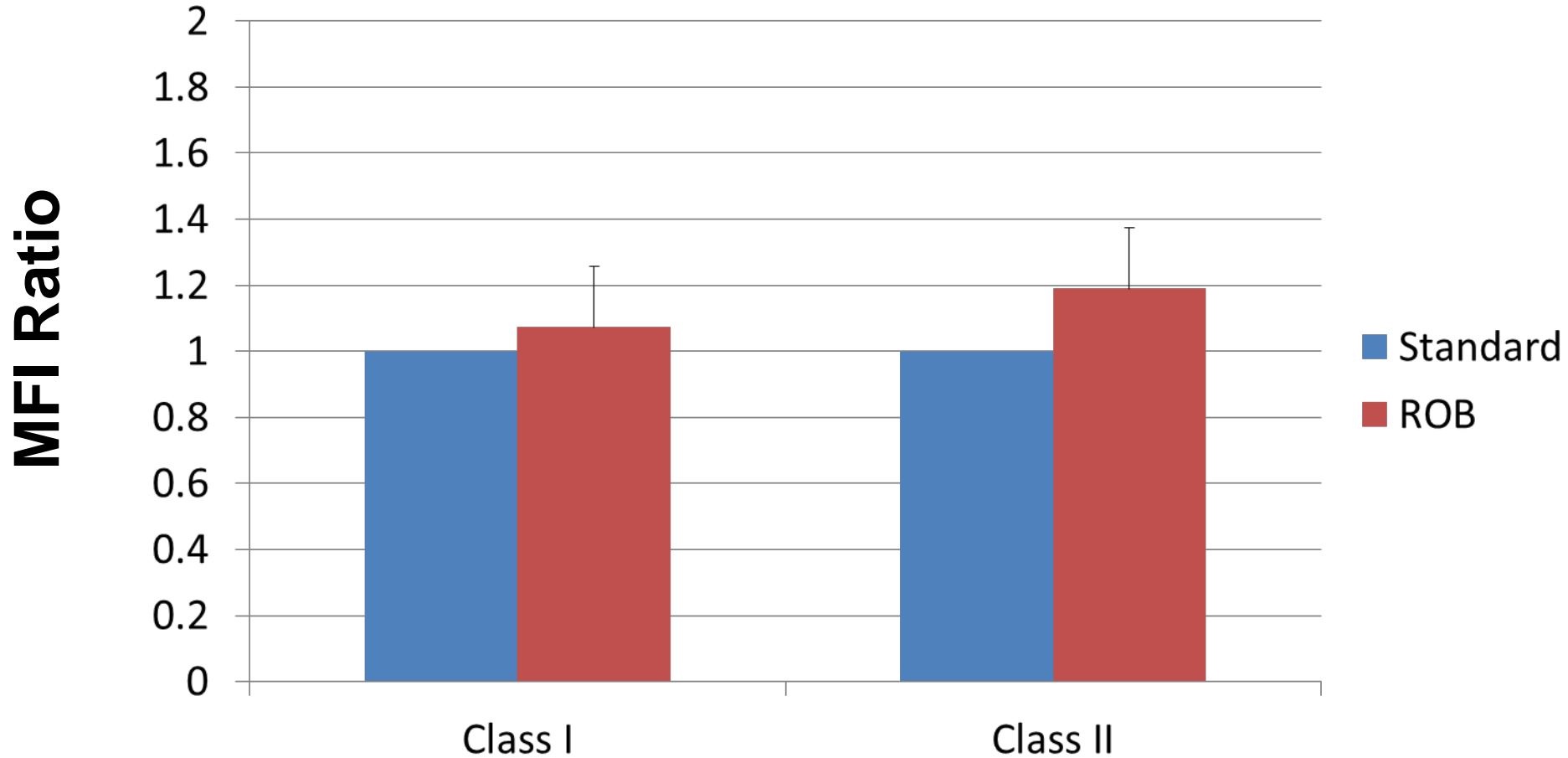


AC-463 Class II



Bead number

Average MFI ratio ROB vs Standard Protocol

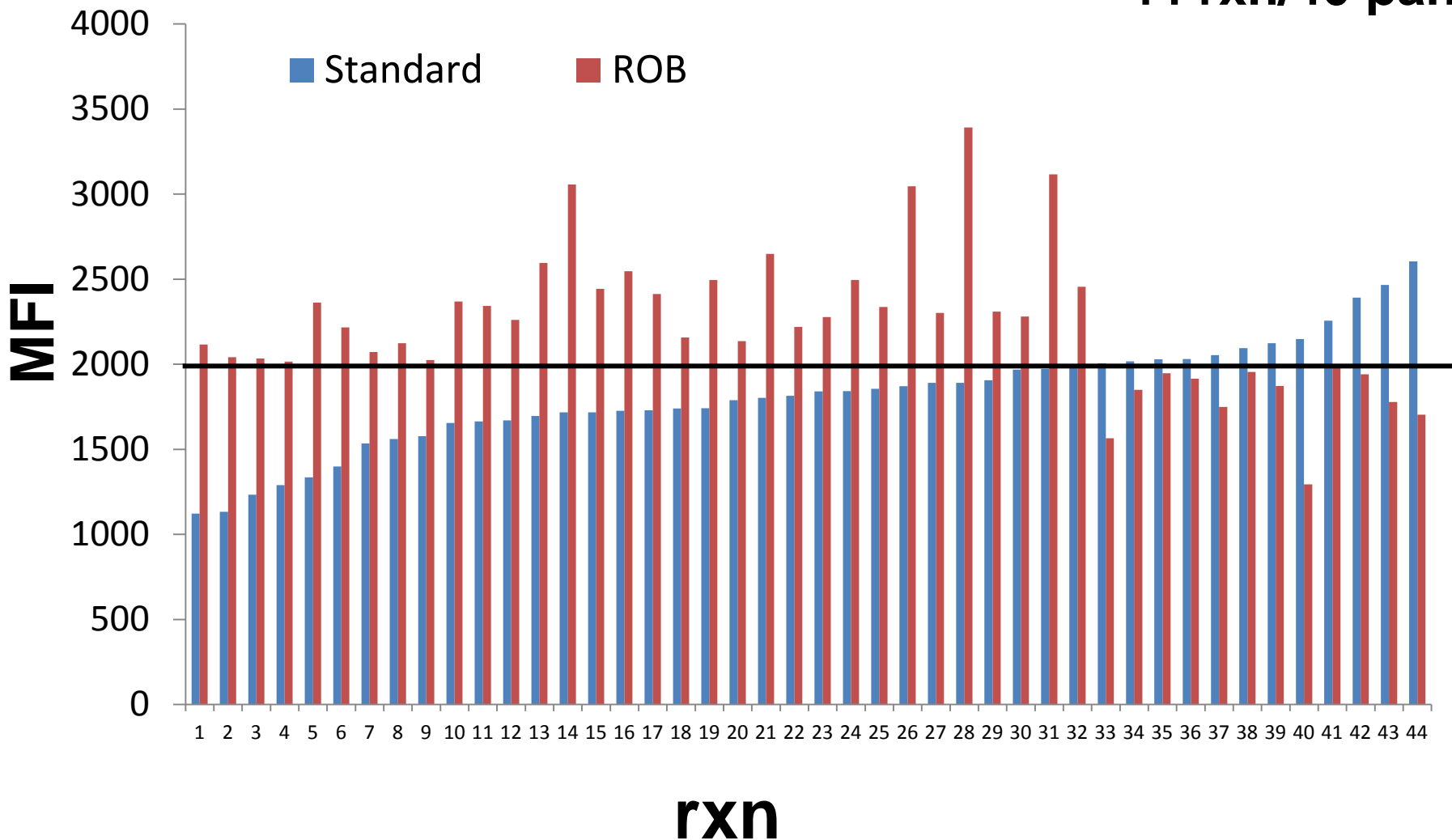


“Discrepant” reactions

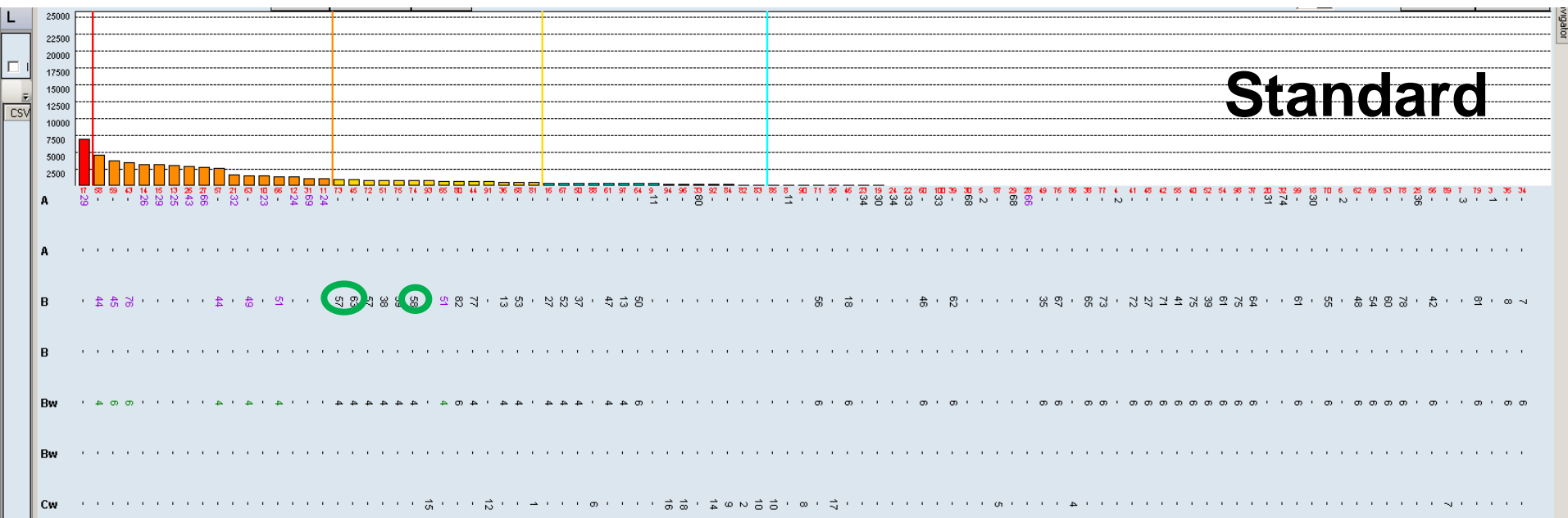
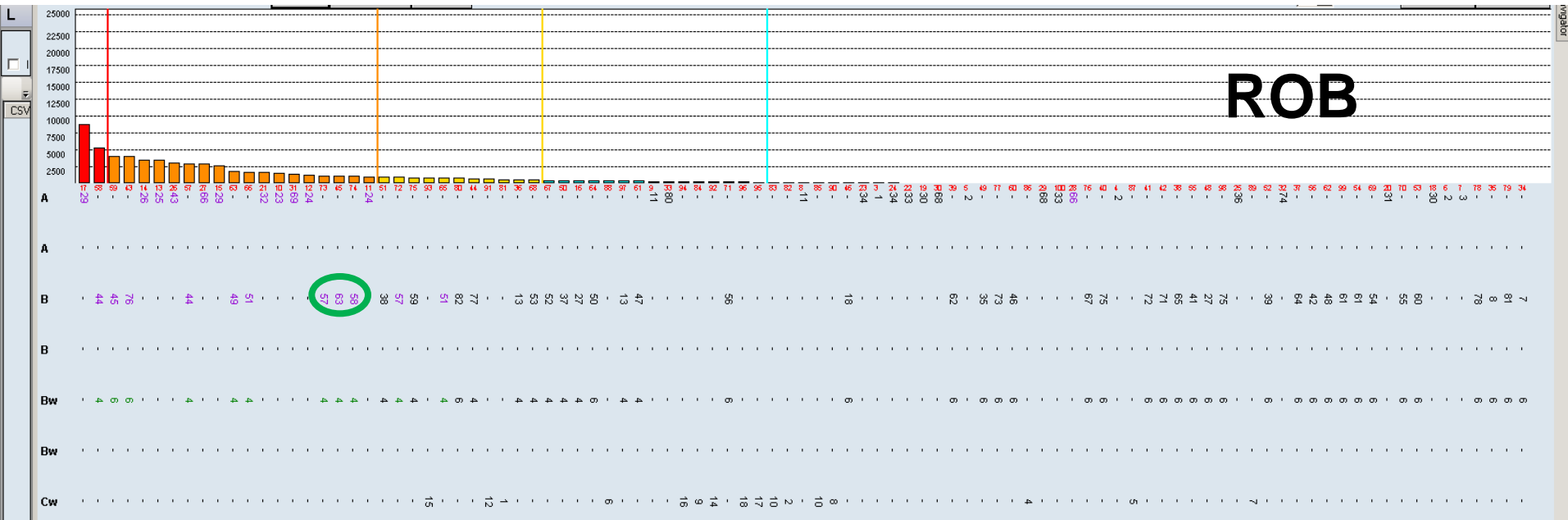
Cut-off 2000 MFI

1.1 rxn/panel

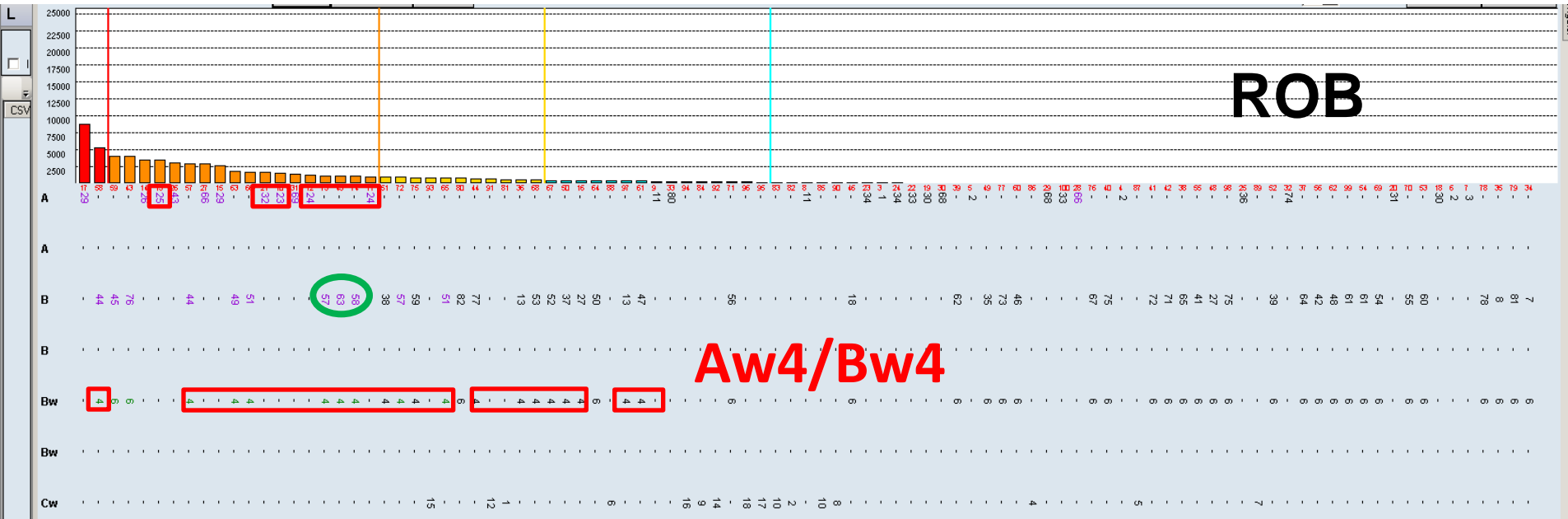
44 rxn/40 panels



Patient serum CF, class I

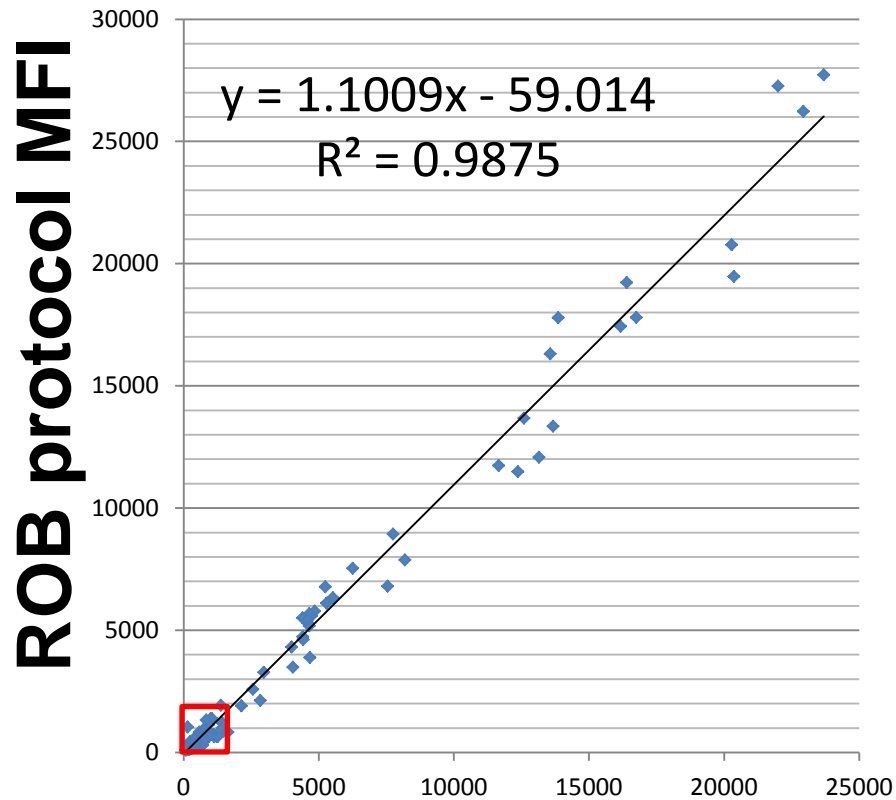


Patient serum CF, class I

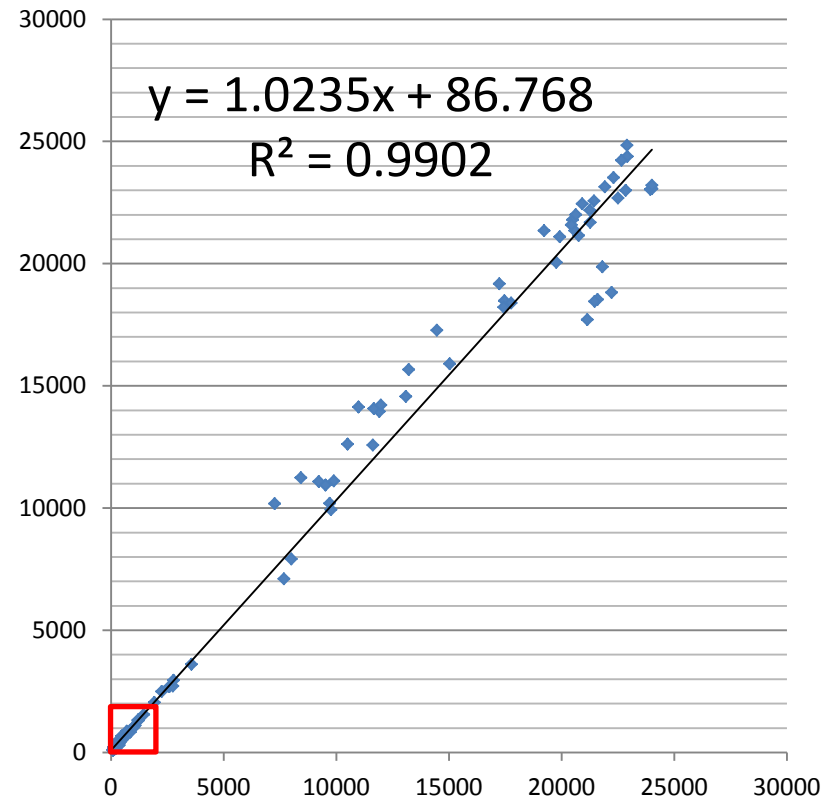


Porto Alegre Laboratory Standard vs ROB MFI correlation, May 2014 ABH PT sera

Class I



Class II



Standard Assay MFI

Conclusion

- We can reduce the time it takes to perform LABScreen[®] SAB Luminex assay without compromising assay sensitivity.
- Correlation between the Standard and ROB protocols is excellent.
- No significant impact on test results when using ROB protocol.
- Significant time and cost saving.
- ROB protocol allows for rapid testing of urgent patient sera.
 - Ex. testing during deceased donor work up.