STRATEGIES TO IMPROVE FREE DRUG TOLERANCE IN ANTI-DRUG ANTIBODY ASSAYS.

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INTRODUCTION

Immunogenicity of biopharmaceutical products is a major concern in clinical and preclinical studies since it can lead to potentially serious side effects, loss of efficacy, and changes in drug exposure, complicating the interpretation of toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) data[1]. As the number of therapeutics with long half-life such as monoclonal antibodies is increasing, drug tolerance in anti-drug antibody (ADA) assays is of growing concern. Most of the techniques that are widely and successfully used for detection and quantification of ADAs in serum or plasma depend on the specific binding of the ADA to its target drug via the antigen binding site. Therefore, only drug-free or partially drug-free ADA can be detected. However, to assess the overall immunogenic potential of a drug, the total amount of ADA would be of particular interest.

Drug tolerance is generally defined as the maximal amount of free drug in the sample that still results in a detectable ADA signal. To compare the drug tolerance of different assays it is usually reported as molar ratio of tolerated free drug to ADA. The tolerated free drug concentration can be interpolated from a titration experiment with an ADA positive control and increasing amounts of free drug, as drug concentration leading to a response equal to assay cut point.

Drug interference for a number of ADA assays has been quantified in our laboratory. These cover a variety of therapeutic drug molecules, platforms, and assay formats.

Table1 Drug tolerance of different ADA assays performed at Celerion.

Type of Drug	Molecular Mass	Half-Life	Assay Type	[Positive Control]	Tolerated [Free Drug]	Molar Ratio of Free Drug/ADA
Fusion Protein	150 kDa	4 days	Bridging ECLA	250 ng/mL	100 ng/mL	0.4
					50'000 ng/mL*	200*
Antibody	150 kDa	4 days	Bridging ELISA	500 ng/mL	19'000 ng/mL	38
Serine Protease	54 kDa	4 hours	Bridging ELISA	100 ng/mL	939 ng/mL	26
Serine Protease	52 kDa	6 hours	Direct ELISA	3060 ng/mL	20'000 ng/mL	19
Peptide	4 kDa	13 hours	RIPA	400 ng/mL	12 ng/mL	1.1

*including acid treatment

The importance of drug tolerance for a particular assay can be estimated based on drug half-life, sampling regime, and drug dosage. In studies with long half-life drugs, repeated and/or high dosage, or short sampling times, interference by the free drug may be an issue and should be analyzed carefully.

Dependent on drug dosage regimen, circulating concentrations of drug in the µg/mL region can be reached. In such cases, molar ratio of drug to ADA should be higher than 100 to be able to detect ADAs at a concentration of 250 ng/mL (the sensitivity that is recommended by the FDA for ADA assays[2]). Table 1 shows that such a level of drug tolerance is usually not reached for standard ADA assays regardless of assay type. Therefore, optimization for drug tolerance is crucial in cases where drug interference is expected to be an issue.

STRATEGIES TO OPTIMIZE FREE DRUG TOLERANCE

In order to demonstrate the impact of a variety of assay parameters on free drug tolerance, two bridging ELISAs have been extensively analyzed in our laboratory. The bridging format is the model of choice as it is most frequently and successfully used to analyze ADAs. Since bridging assays rely on the availability of both antigen binding sites on ADAs, only drug-free ADAs can be detected, rendering bridging assays especially susceptible to the presence of free drug. However, a well-chosen assay setup can substantially reduce drug interference.

Acid Treatment of Samples

Acid treatment of samples has been successfully used to improve free drug tolerance in ADA assays. Antibody-antigen binding is weakened and eventually disrupted at low pH, making the detection of partially or completely drug-bound ADAs in bridging ELISAs possible.

Figure 1 Schematic representation of the standard assay format with or without acid-treatment of samples

The acid treatment results in a dissociation of drug-ADA complexes, making the detection of partially or completely drug-bound ADAs possible.
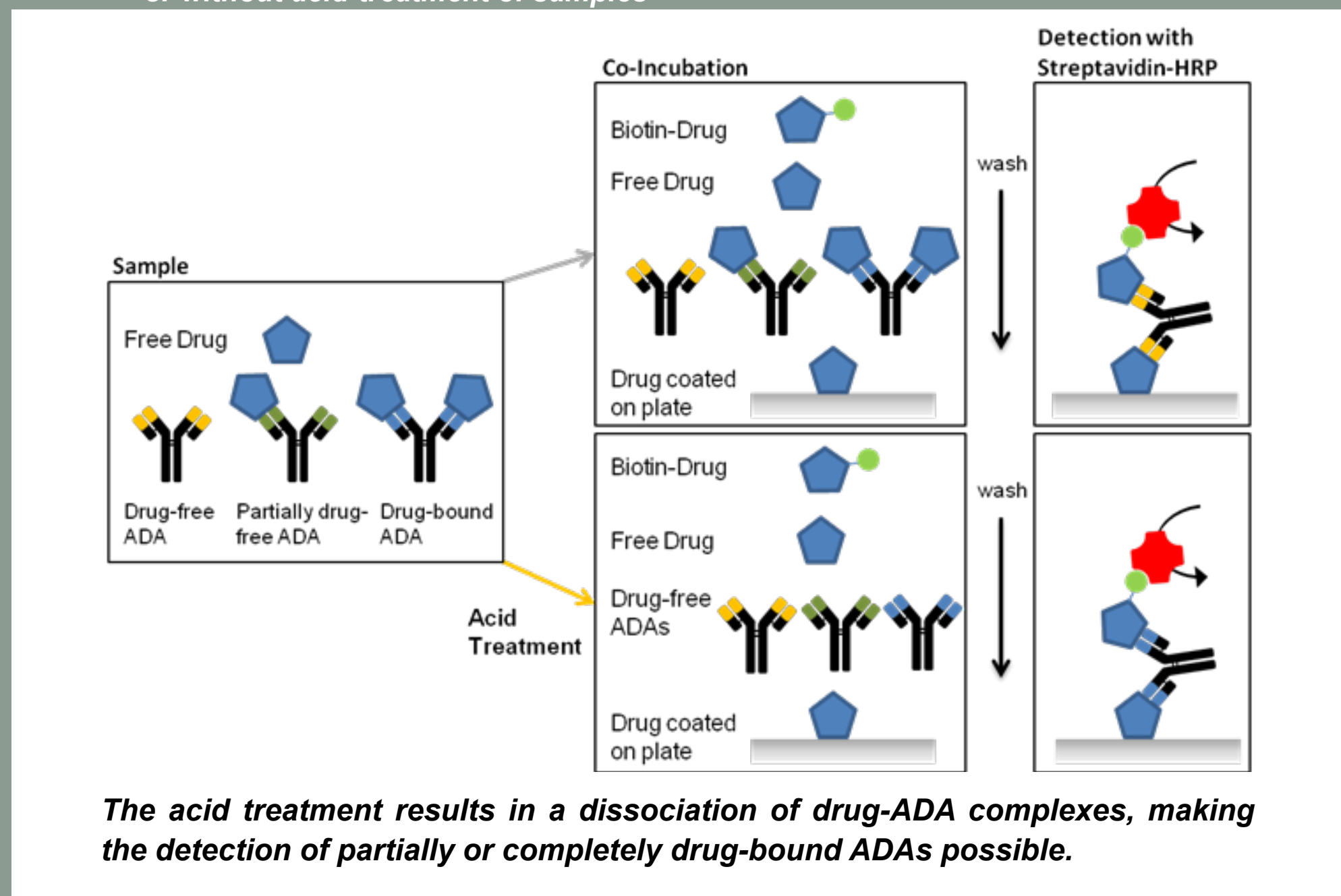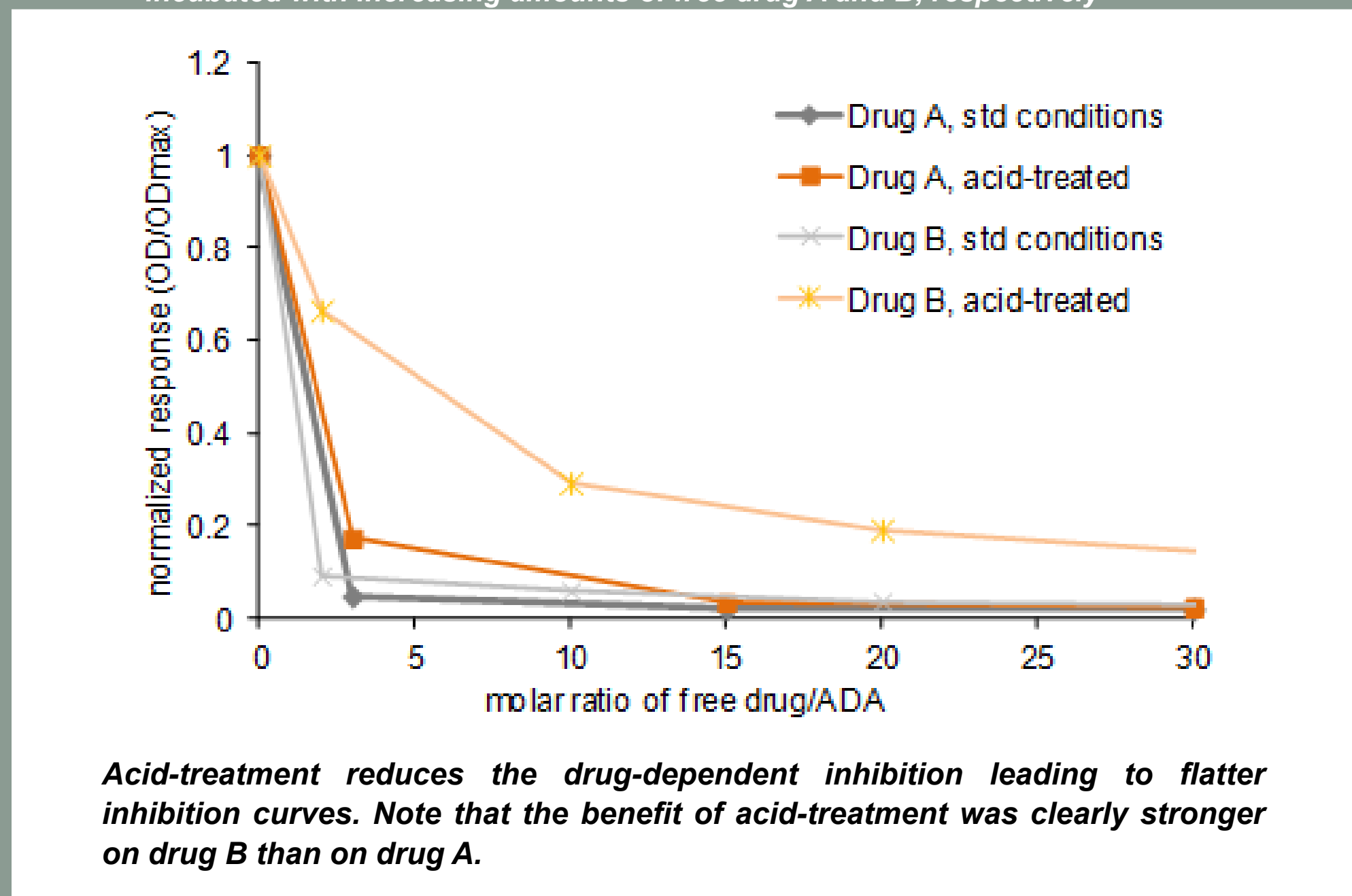

Figure 2 Normalized responses of controls containing anti-drug A and anti-drug B antibodies incubated with increasing amounts of free drug A and B, respectively
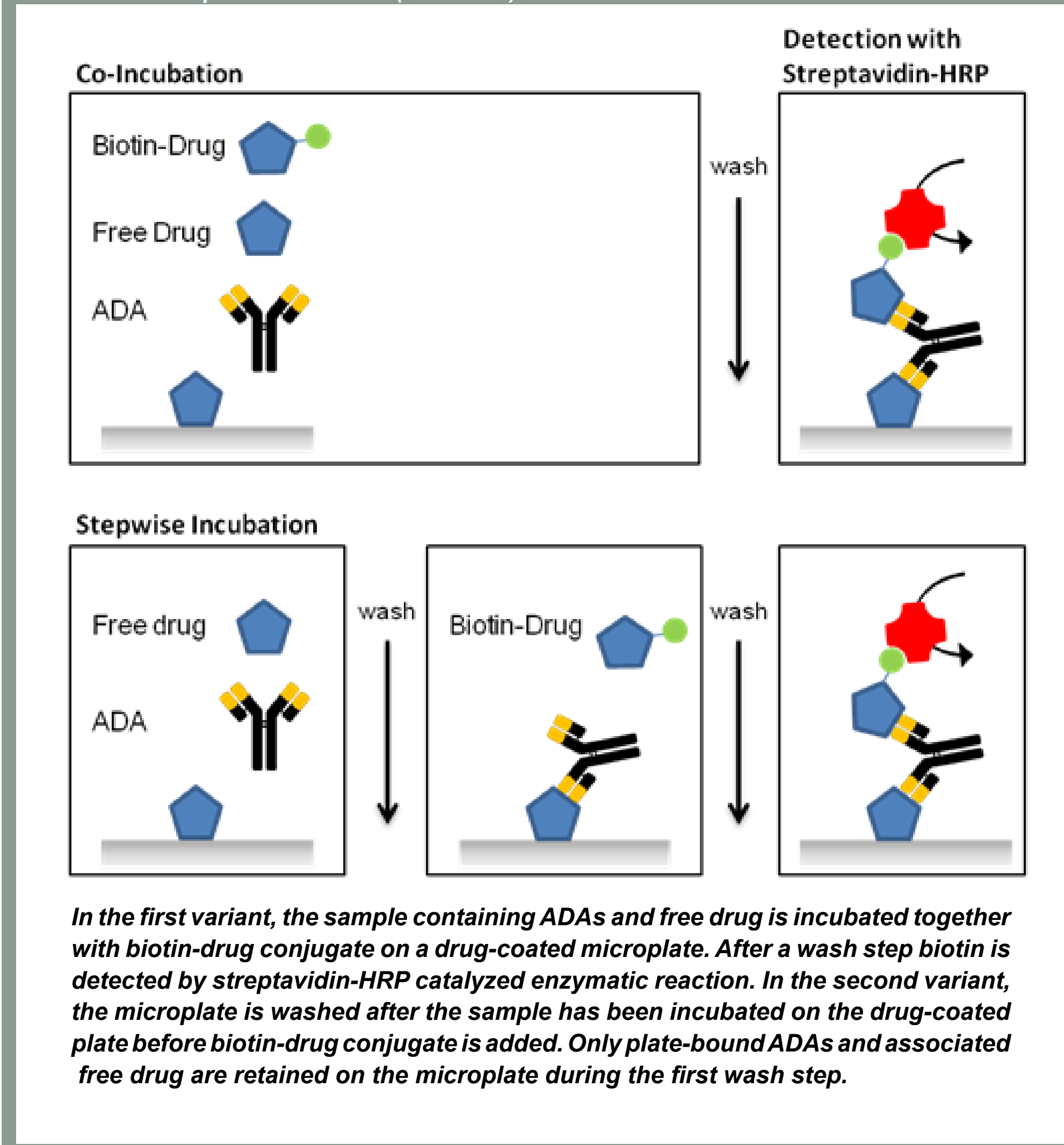
Acid-treatment reduces the drug-dependent inhibition leading to flatter inhibition curves. Note that the benefit of acid-treatment was clearly stronger on drug B than on drug A.

Acid treatment generally increased drug tolerance (Figure 2). However, the magnitude of the improvement greatly depended on the specific assay, even though initial setup and drug tolerance were similar. For drug A drug tolerance was increased from 26 to 43 whereas for drug B it was increased from 38 to 159. Acid-treatment without further optimization of the assay may not in all cases lead to a clear improvement of drug tolerance. Other parameters such as assay format or labeling conditions should also be taken into account.

Optimization of Assay Design

Figure 3 Schematic representation of assays with a co-incubation (upper row) or a stepwise incubation (lower row)

In the first variant, the sample containing ADAs and free drug is incubated together with biotin-drug conjugate on a drug-coated microplate. After a wash step biotin is detected by streptavidin-HRP catalyzed enzymatic reaction. In the second variant, the microplate is washed after the sample has been incubated on the drug-coated plate before biotin-drug conjugate is added. Only plate-bound ADAs and associated free drug are retained on the microplate during the first wash step.

In a first step, single assay parameters were changed and the effect on assay drug tolerance was analyzed. Standard assay was conducted under a co-incubation setup (Figure 3, top). This format was also evaluated using biotin-drug at a higher concentration or an improved biotin-drug conjugate with an increased rate of biotin incorporation. In addition, a stepwise assay setup (Figure 3, bottom) was also tested.

Figure 4 Evaluation of single assay parameters change on free drug tolerance The molar ratio of free drug/ADA is indicated for each condition on top of the bars
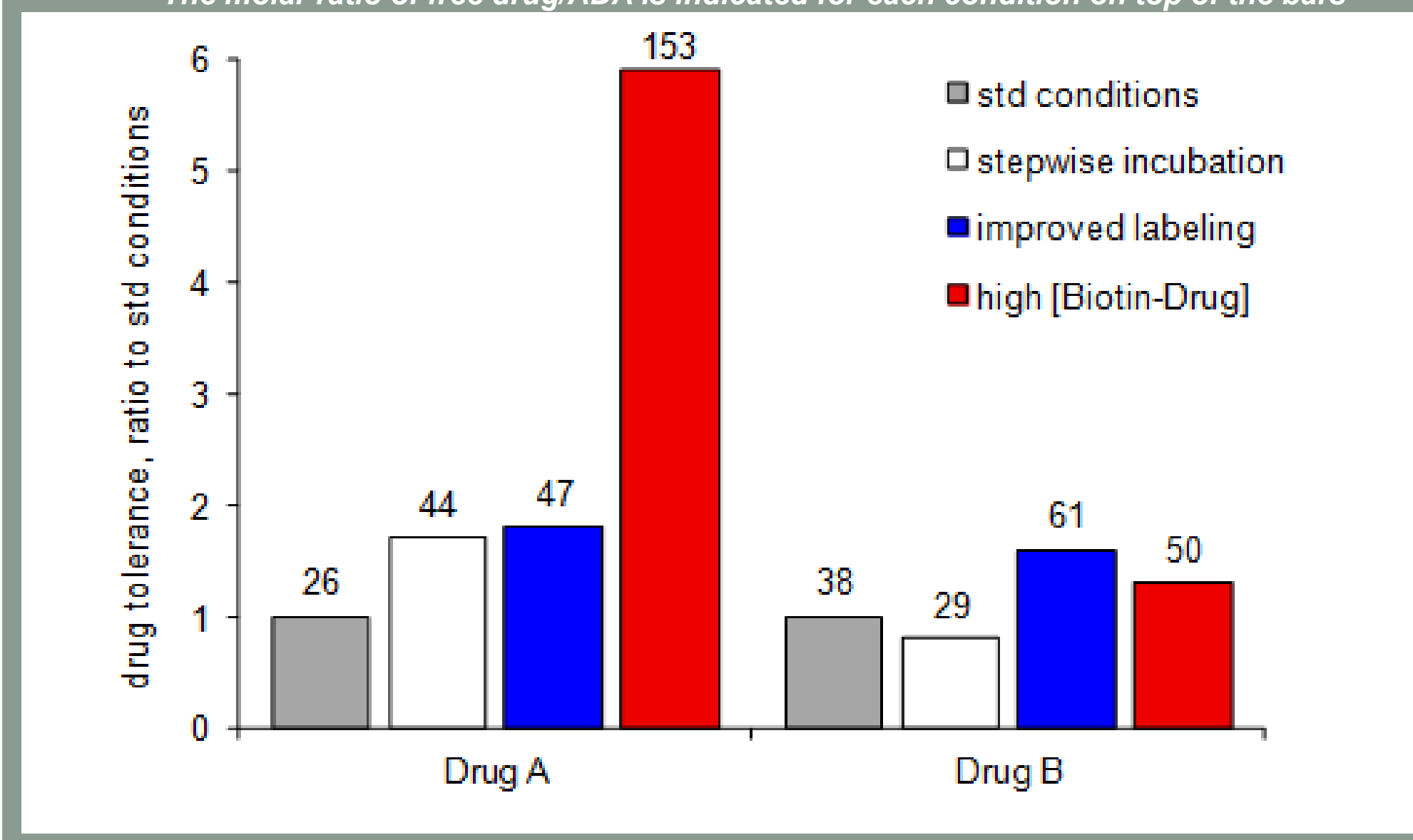
These single changes in assay parameters had contrasting outcomes on drug tolerance between the 2 drugs. For drug B all modifications tested resulted at best in a marginal improvement in drug tolerance. Results were better overall for drug A, and the use of a higher concentration of biotin-drug was sufficient to give a free drug/ADA ratio above the 100 threshold defined above.

The stepwise assay format (Figure 3) was then systematically evaluated in combination with both biotin-drug conditions defined in Figure 4 for further improvement in free drug tolerance.

Figure 5 Evaluation of combined assay parameters changes on free drug tolerance The molar ratio of free drug/ADA is indicated for each condition on top of the bars
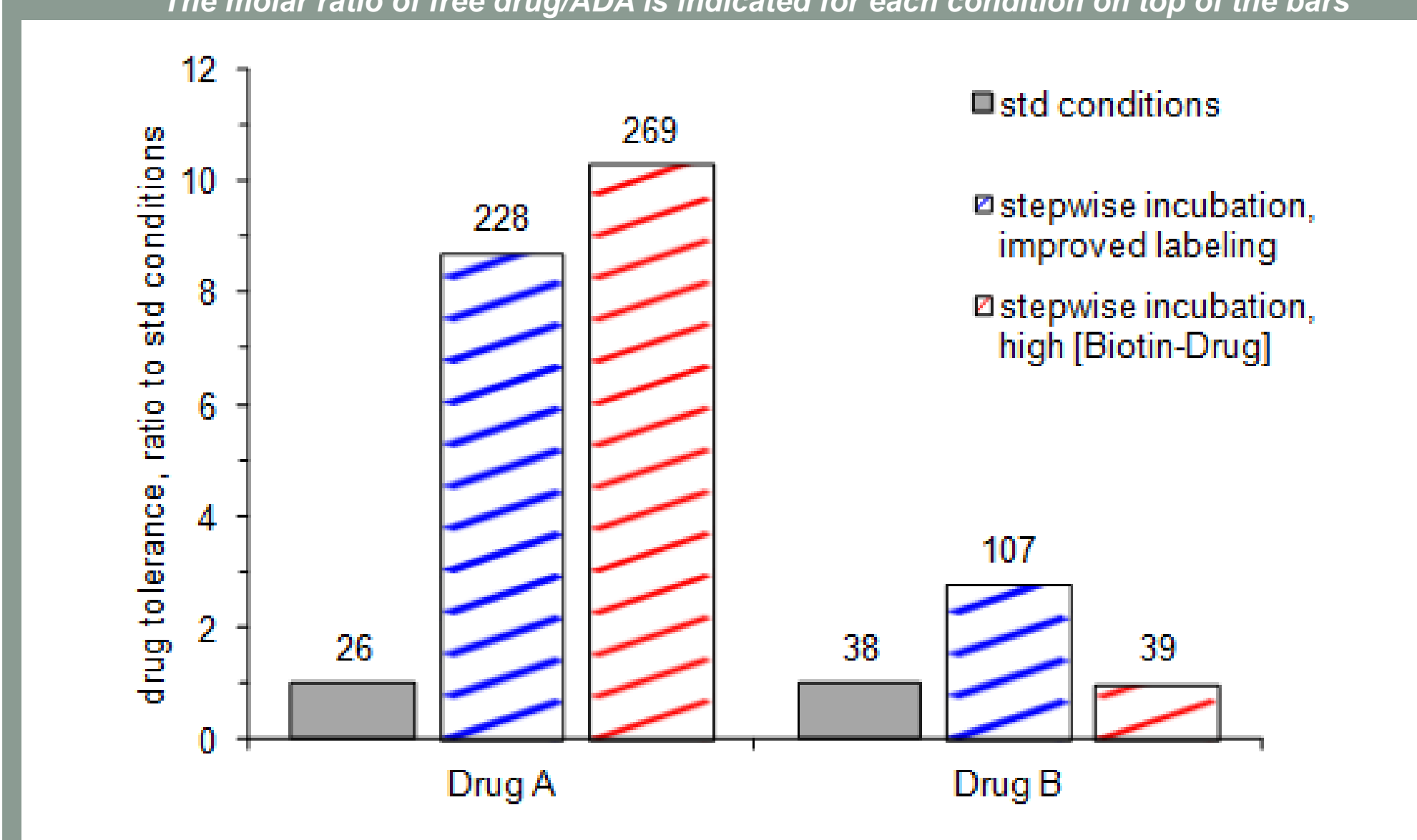
A cumulative improvement in free drug tolerance was observed for drug A under both biotin-drug conditions when using the stepwise format, and in both cases the free drug/ADA ratio measured was well above the 100 threshold. The optimal assay design for free drug tolerance identified here for drug A combines a stepwise incubation with use of a higher concentration of biotin-drug conjugate.

A similar but less dramatic cumulative improvement in free drug tolerance was observed for drug B only when the stepwise incubation was combined with use of the improved biotin-drug conjugate; this assay design was selected as optimal for drug B as it also yielded a free drug/ADA ratio above the 100 threshold.

Taken together these results demonstrate that under certain conditions careful assay setup may be enough to obtain acceptable levels of free drug tolerance.

Combined Effects

Finally, optimized assay design identified in Figure 5 were used in combination with acid treatment of samples and compared to standard assay conditions.

Figure 6 Evaluation of acid treatment together with optimized assay design The molar ratio of free drug/ADA is indicated for each condition on top of the bars
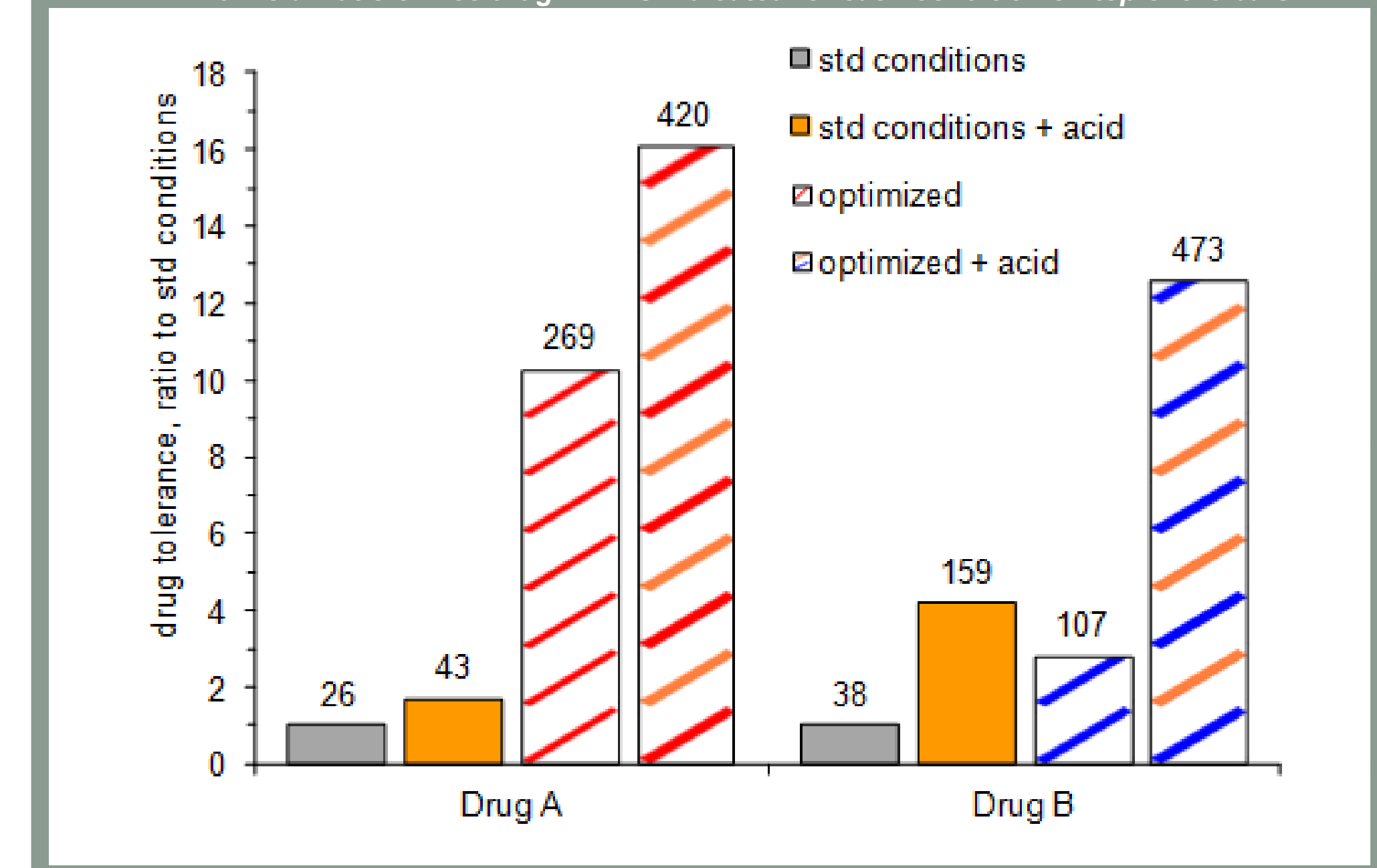
Acid sample pre-treatment had a multiplier effect on free drug tolerance that was drug dependent but not assay design dependent. For drug A a relatively modest improvement of drug tolerance of 1.6 fold was observed in both cases. For drug B the improvement was more dramatic at around 4 fold the level without acid treatment for both assay designs evaluated. Overall, combining the optimized assay format with acid treatment resulted in an increase in drug tolerance of 16 fold and 12 fold for drug A and drug B, respectively.

CASE STUDY

Rat serum samples taken from four-week repeated dose toxicology studies were analysed for ADAs in a ECL bridging assay at week 0 (pre-dose samples), week 2, 3, and 4. The drug was administrated twice weekly at two dose levels. Samples from a first study were not pre-treated, whereas samples from a second follow-up study were acid-treated.

Figure 7 Rate of ADA positive samples in rats treated with low (top panel) and high dose (bottom panel) of drug
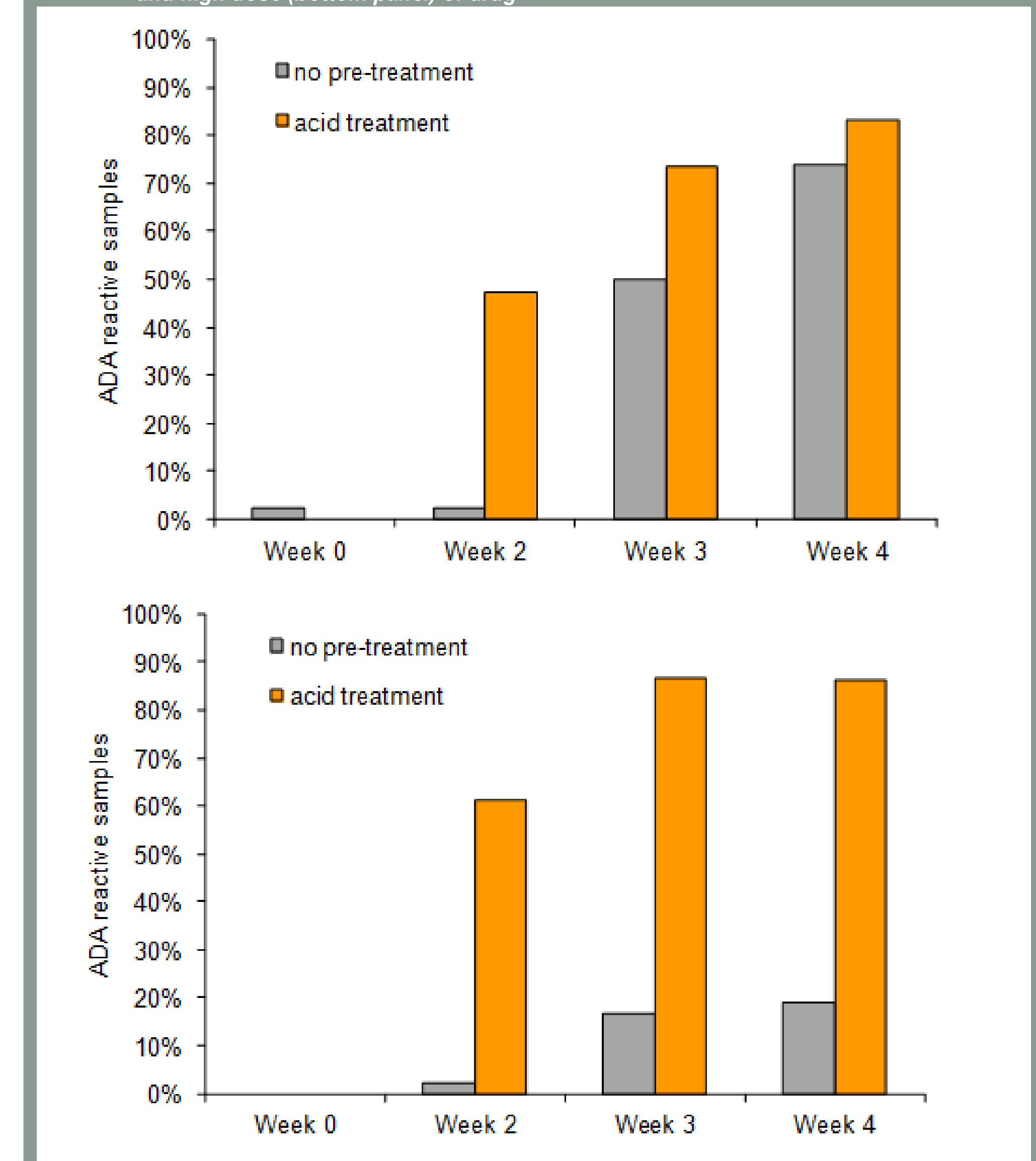
Acid-treatment generally increased the rate of ADA positive samples, indicating that drug interference in untreated samples inhibits specific assay response and increases the rate of not-detected ADA positive samples (false negative).

In the low-dosed rats the difference between non-treated and acid-treated samples disappeared after 3 to 4 weeks of treatment, suggesting that high antibody titers at these time points may overcome the drug-dependent assay inhibition. In the high-dosed rats this effect was not observed, indicating that the higher amount of circulating drug more efficiently interfere with detection of ADAs when the samples are not acid-treated.

Together, poor free drug tolerance in untreated samples lead to a high rate of false negative antibody results, potentially affecting the conclusions drawn from this study.

DISCUSSION AND CONCLUSION

Drug interference can be a major challenge in immunogenicity assays and should be considered carefully at the beginning of assay development. We propose to use a risk-based approach to evaluate whether drug interference is an issue for determination of a particular ADA response. Study parameters such as dosage and sampling regime as well as drug half-life should be taken into account to estimate the need for improved assay drug tolerance.

Acid treatment is the most straightforward and effective strategy to improve free drug tolerance. However, in certain cases, the effect of acid treatment may be only marginal in absence of further assay optimization. In addition, acid treatment is work-intensive and a possible source of variation and analytical errors, impacting assay robustness. Therefore, we suggest including controls for drug tolerance early in assay development and optimizing assay parameters accordingly.

REFERENCES

1 Koren E. et al., J Immunol Meth, 2008, 333, 1-9
2 Draft Guidance, Assay Development for Immunogenicity Testing of Therapeutic Proteins, U.S. Food and Drug Administration, 2009

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