PCA技术第4应用领域：MouseBridge和RabBridge PCA ELISA用于关键抗体试剂的质量控制

1. ICH生物分析方法验证M10第4部分：配体结合测定（LBA），2019年2月26日批准

Topics included in ICH M10 guideline:

1. Definition of critical reagents.

2. Critical reagents throughout the lifecycle of the assay:
   (1.) Lot-change.
   (2.) Long-term stability.

3. Documentation.

在测定的整个生命周期内使用关键试剂

确保研究样品中的分析物浓度在测定的使用寿命内具有可比性

Validation

- ➢Change of critical reagents
  - –Run partial validation, if required
    - ➢Monitoring of the assay over time
  - –Including long-term stability
    - ➢Ongoing study sample analysis

关键试剂的定义 （ICH M10）
Definition of Critical Reagents (ICH M10)

对于配体结合测定（LBA），关键试剂是参与结合和染色反应的（通常是生物）试剂/分子，即使不被注意也可以改变测定结果。 因此，这些试剂会影响LBA测定的有效性。

因此，在将试剂用于样品分析之前，关键试剂的批次切换需要额外的桥接/鉴定实验。

关键试剂的批次更换 – 1
Lot-change of critical reagents – 1

微小的试剂变化被定义为那些预计对测定性能影响最小的试剂变化，因此可以实施而不会对数据生成产生任何有害影响。

Examples:

- new reagent lot derived from a previously qualified stock
  - such as a new purification of polyclonal sera from the same animals
  - a new conjugation using the same protein lot when the conjugation process has been demonstrated to be well controlled

关键试剂的批次更换 – 2

主要变化：“这是最广泛的试剂认证级别，主要针对更换关键试剂，其中试剂的原始来源不再可用”

Examples:

- antibody lots obtained from new animals,
- new clones for monoclonal antibody production, or
- new cell lines for the generation of recombinant material.

ICH M10 关键试剂部分未包含的主题

- Reference standard
  - ➢Not classed in scope as critical reagent for PK assay
- Selection of critical reagents
  - ➢Part of assay development, which is not part of the guideline
- Level of characterization of critical reagents
  - ➢Risk based approach
  - ➢Different possibilities for commercial available and customized reagents

EMA （2011）： 7.1.1.12.关键试剂定义：

- **Critical reagents, including binding reagents** (e.g. binding proteins, aptamers, antibodies or conjugated antibodies) **and those containing enzymatic moieties have direct impact** on the results of the assay and therefore **their quality must be assured**. Accordingly, when **changing reagent batches** during validation or sample analysis the analytical **performance of the method must be verified** to ensure that it is not altered compared with the original or previous batch.

- **Conditions guaranteeing the maintenance of the stability** of both non critical reagents (e.g. buffers, diluents or acidification reagents) and more importantly of the critical reagents should be documented in order to ensure that the performance of the method is not affected over time.

FDA（2013年草案）：关键试剂定义：

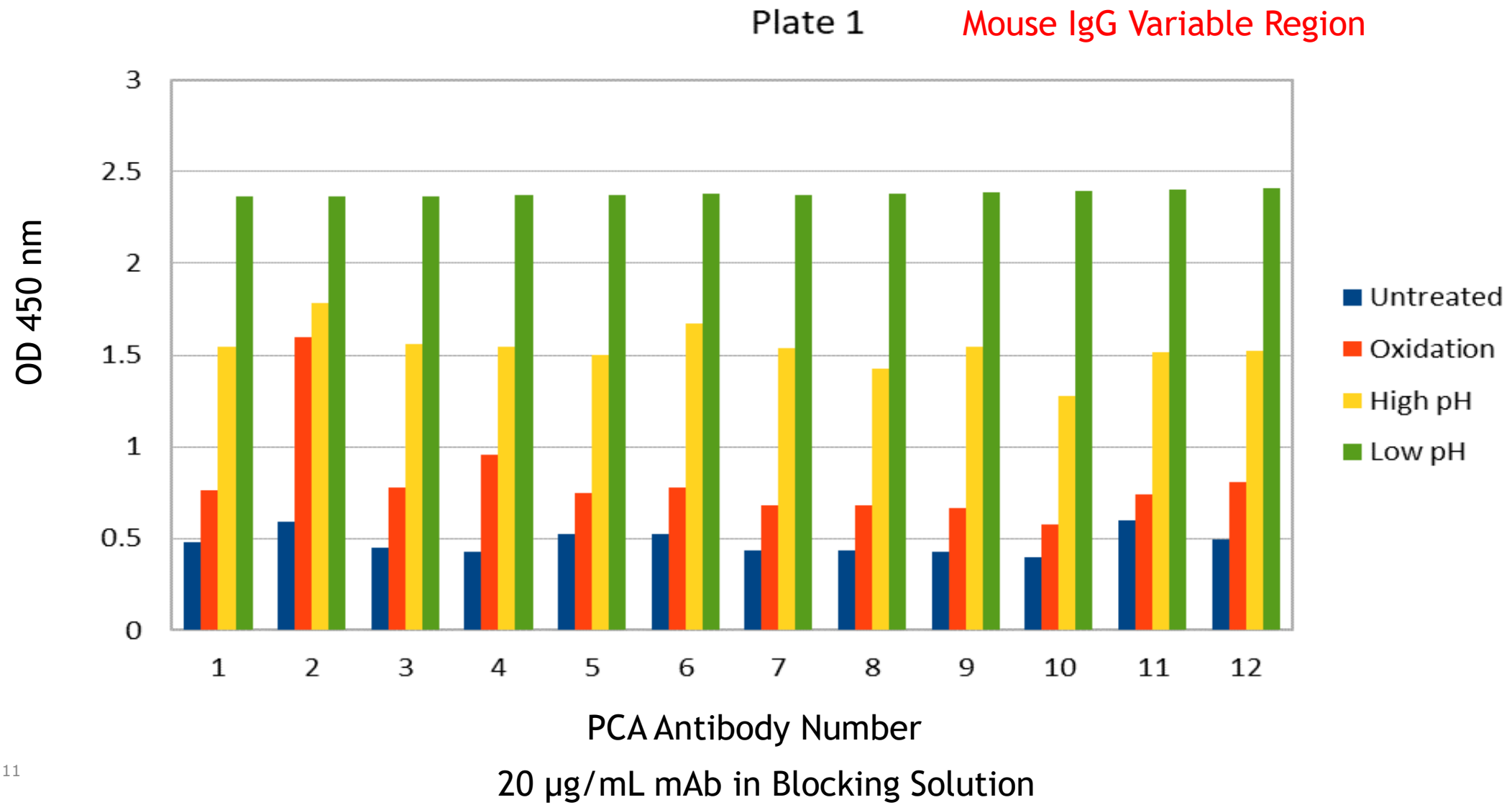
Key reagents, such as reference standards, antibodies, tracers, and matrices should be characterized appropriately and stored under defined conditions. Assay reoptimization or validation may be important when there are changes in key reagents.

日本（LBA，2013）：6.5。关键试剂定义：

1. A critical reagent is the one that has a direct impact on the results of an LBA-based bioanalytical method and usually includes, but is not limited to, binding reagents (e.g., unlabeled or labeled antibodies).

2. A critical reagent should be selected by considering the specificity for the analyte and should be stored under conditions that ensure consistent quality. The quality of critical reagent should be appropriately maintained throughout the period of use in analytical method validation and study sample analysis. Partial validation is in principle required when the critical reagent lot is changed.

案例1.PCA 对小鼠单克隆抗体 HOS 状态的敏感性



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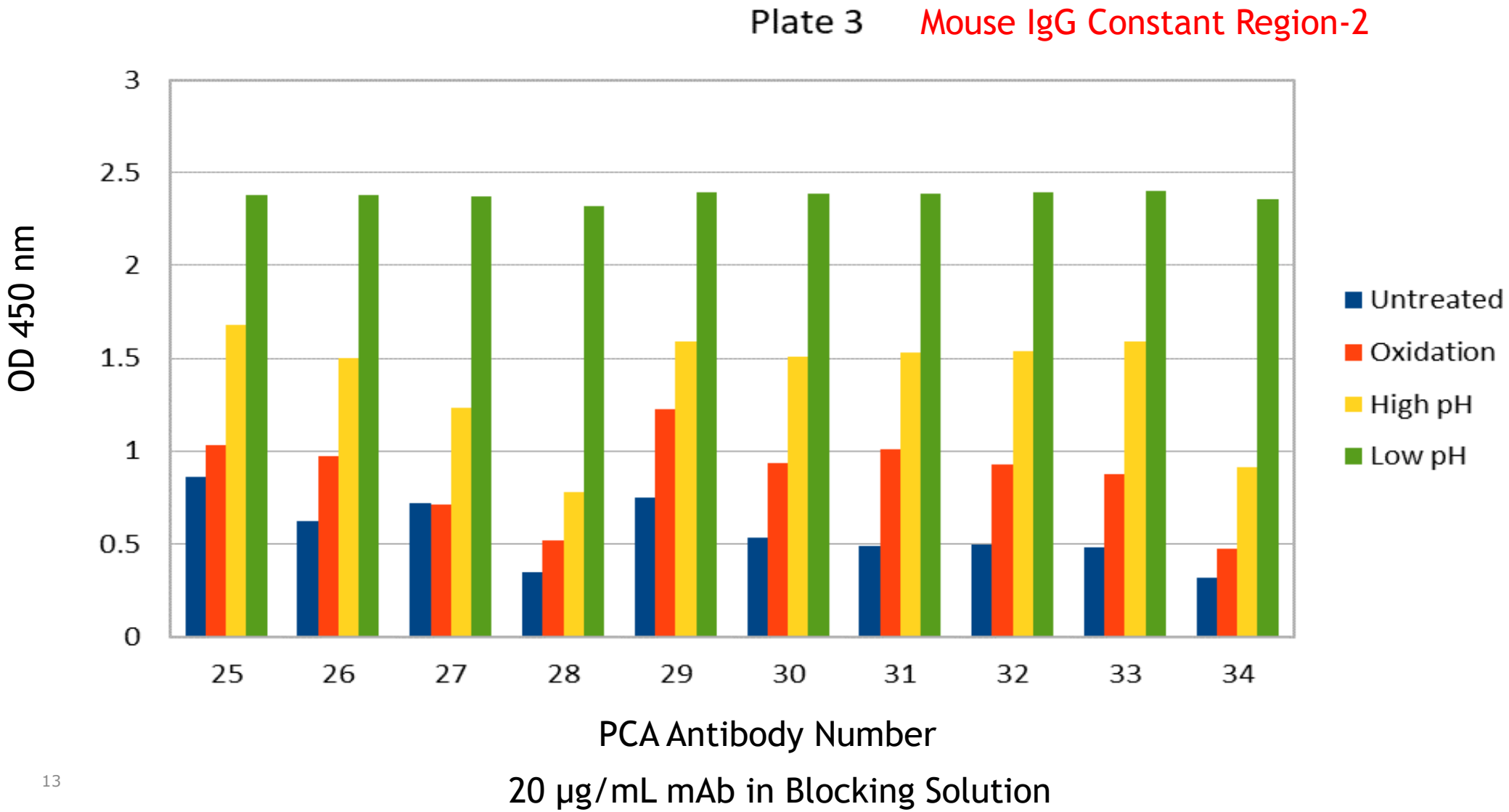
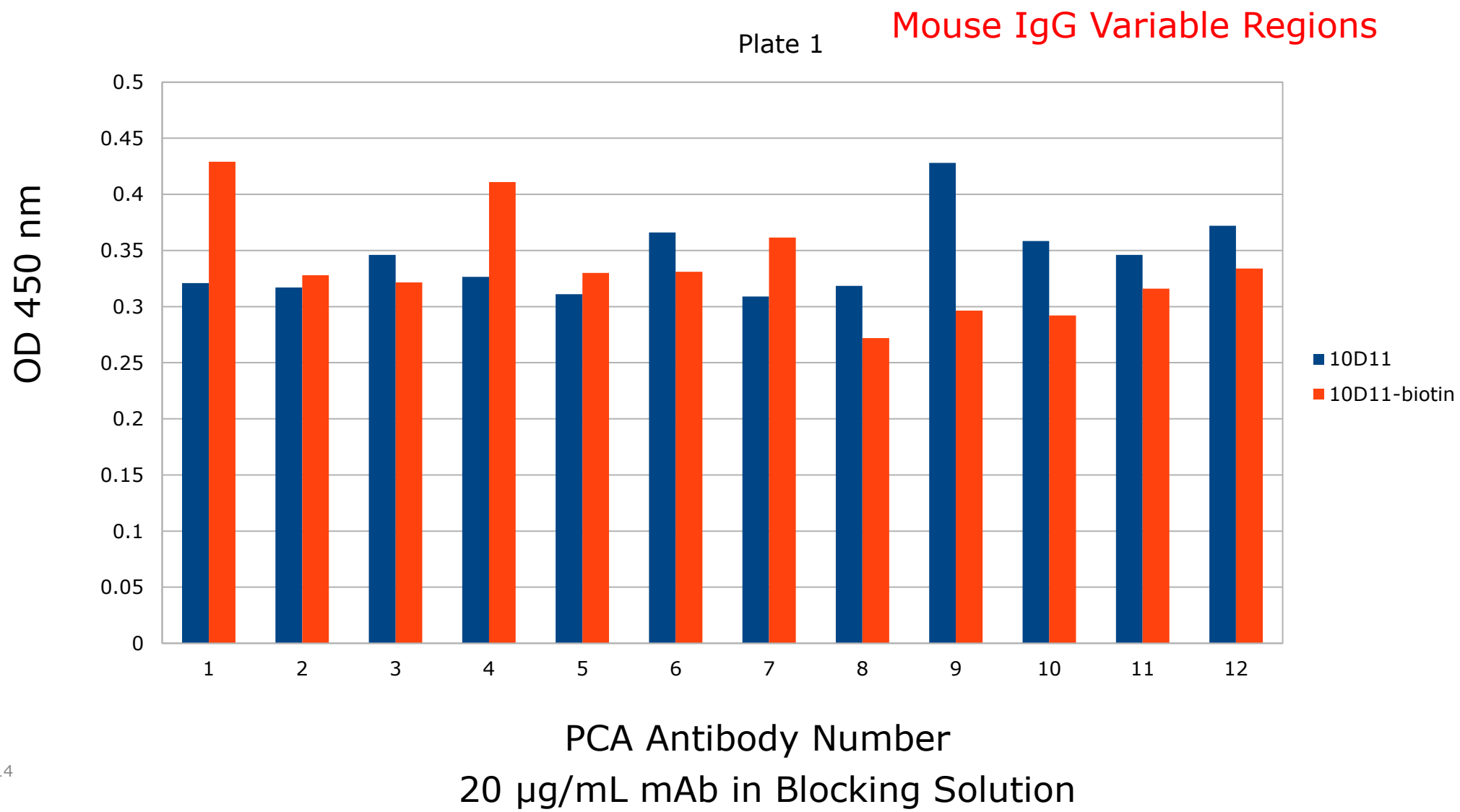


案例2. 单克隆抗体生物素化对其HOS状态的影响



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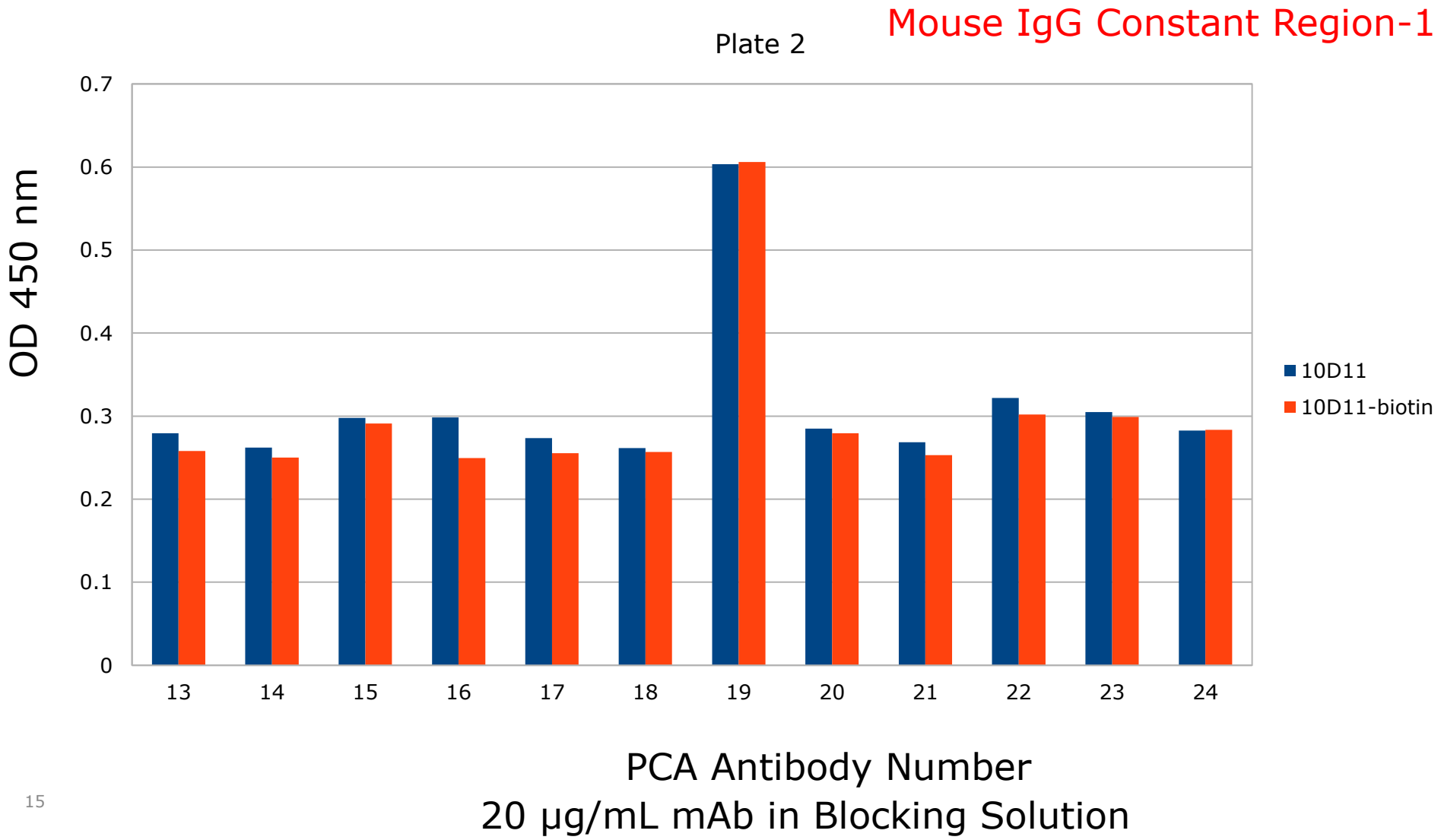

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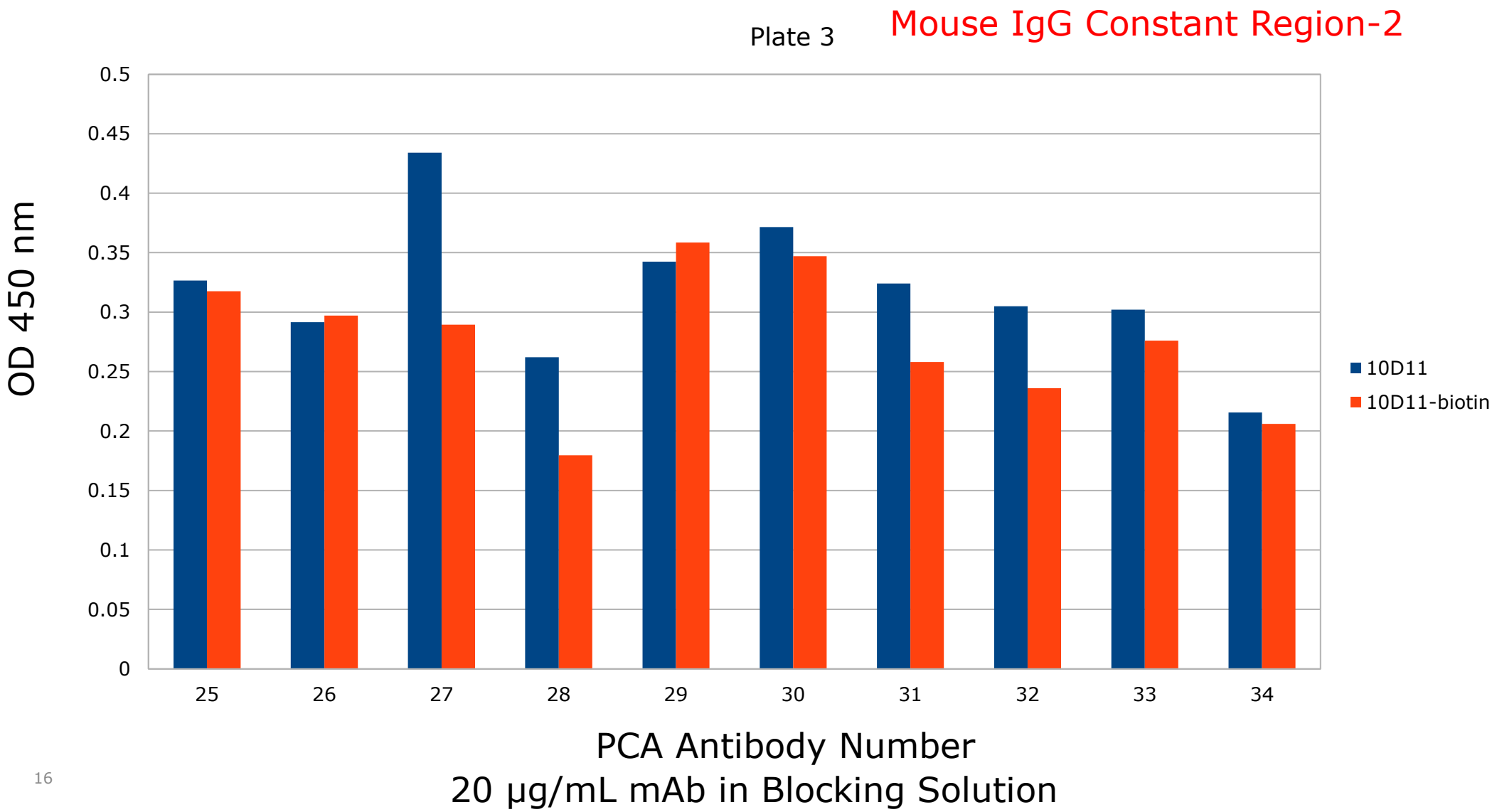
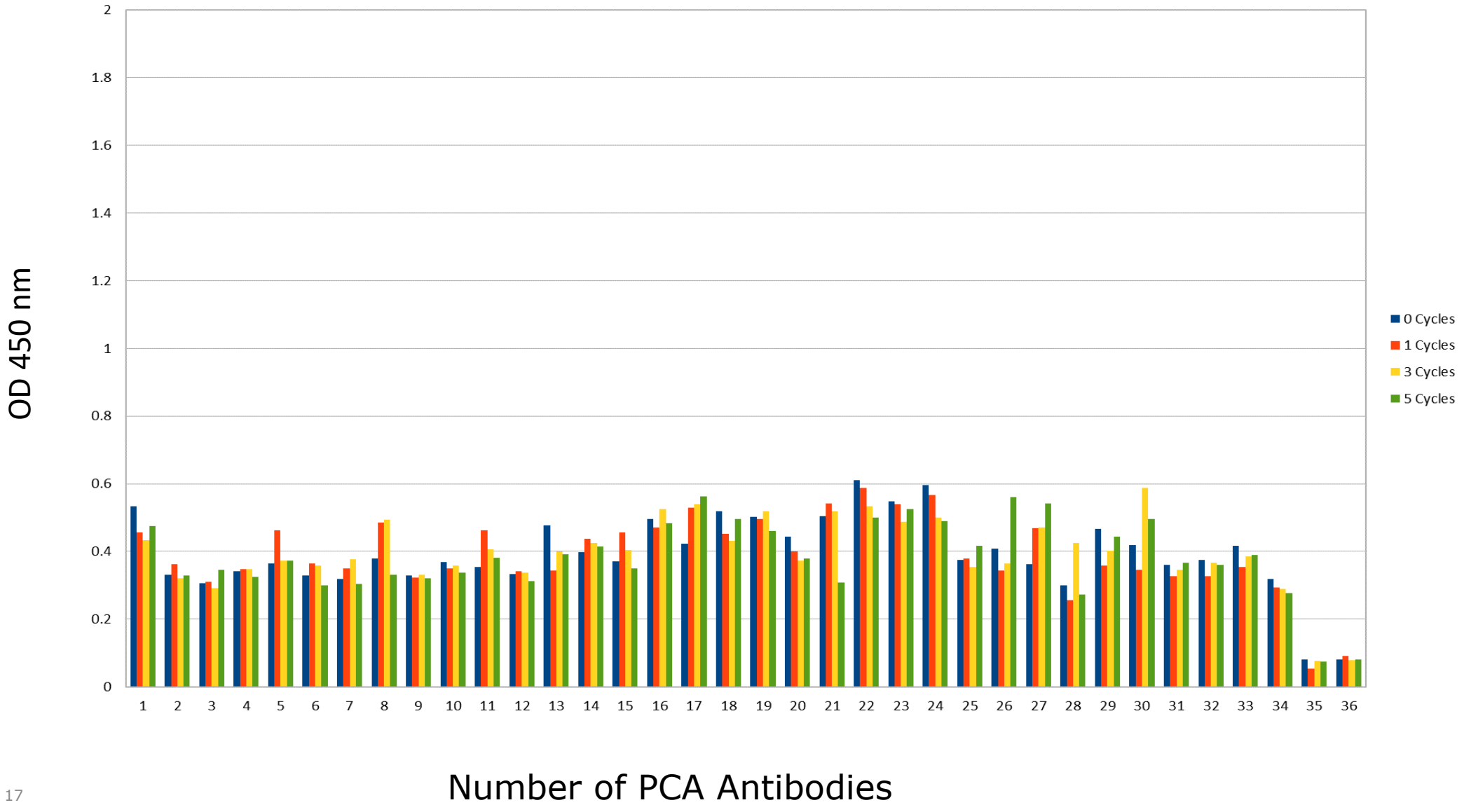
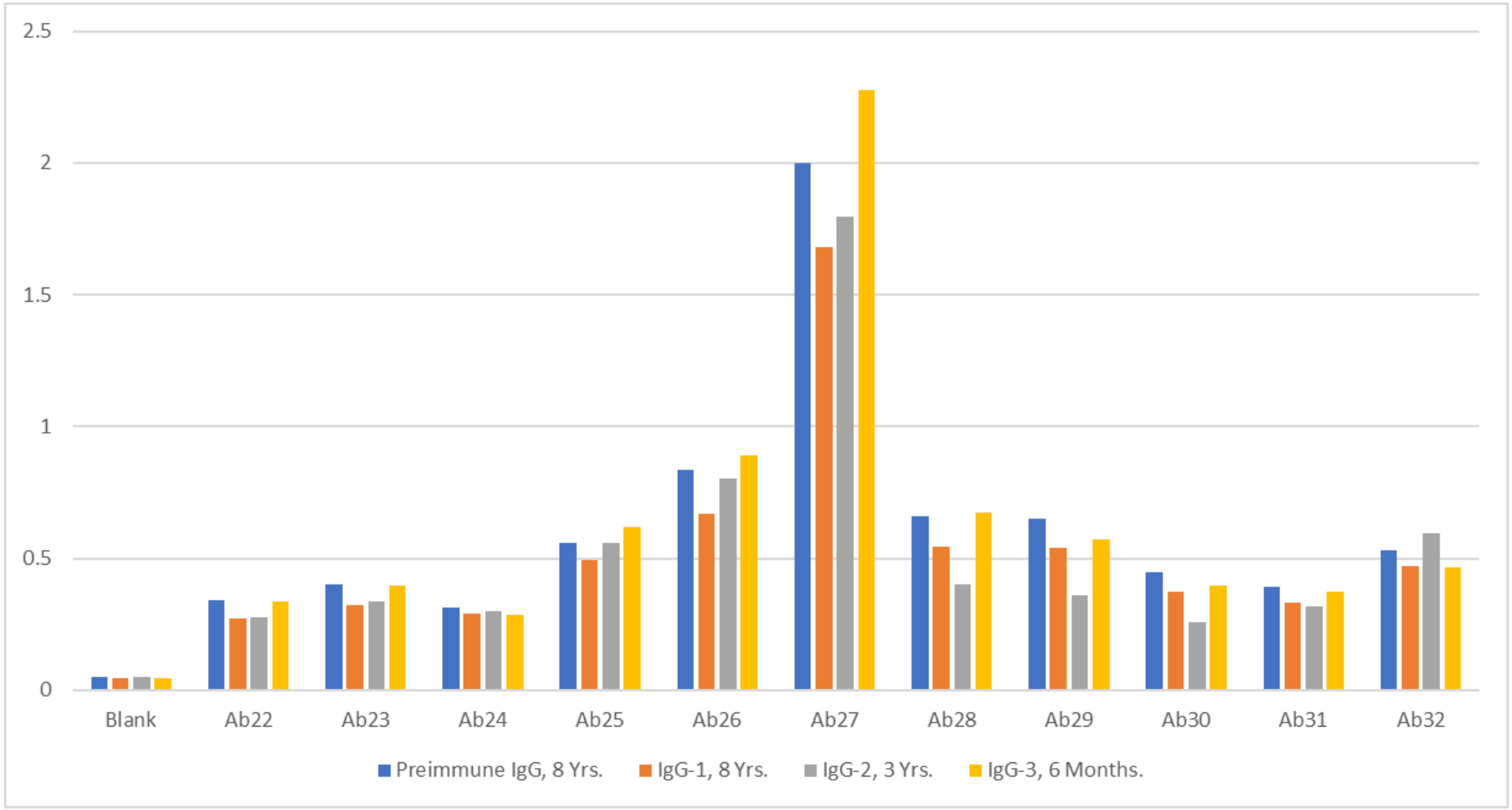


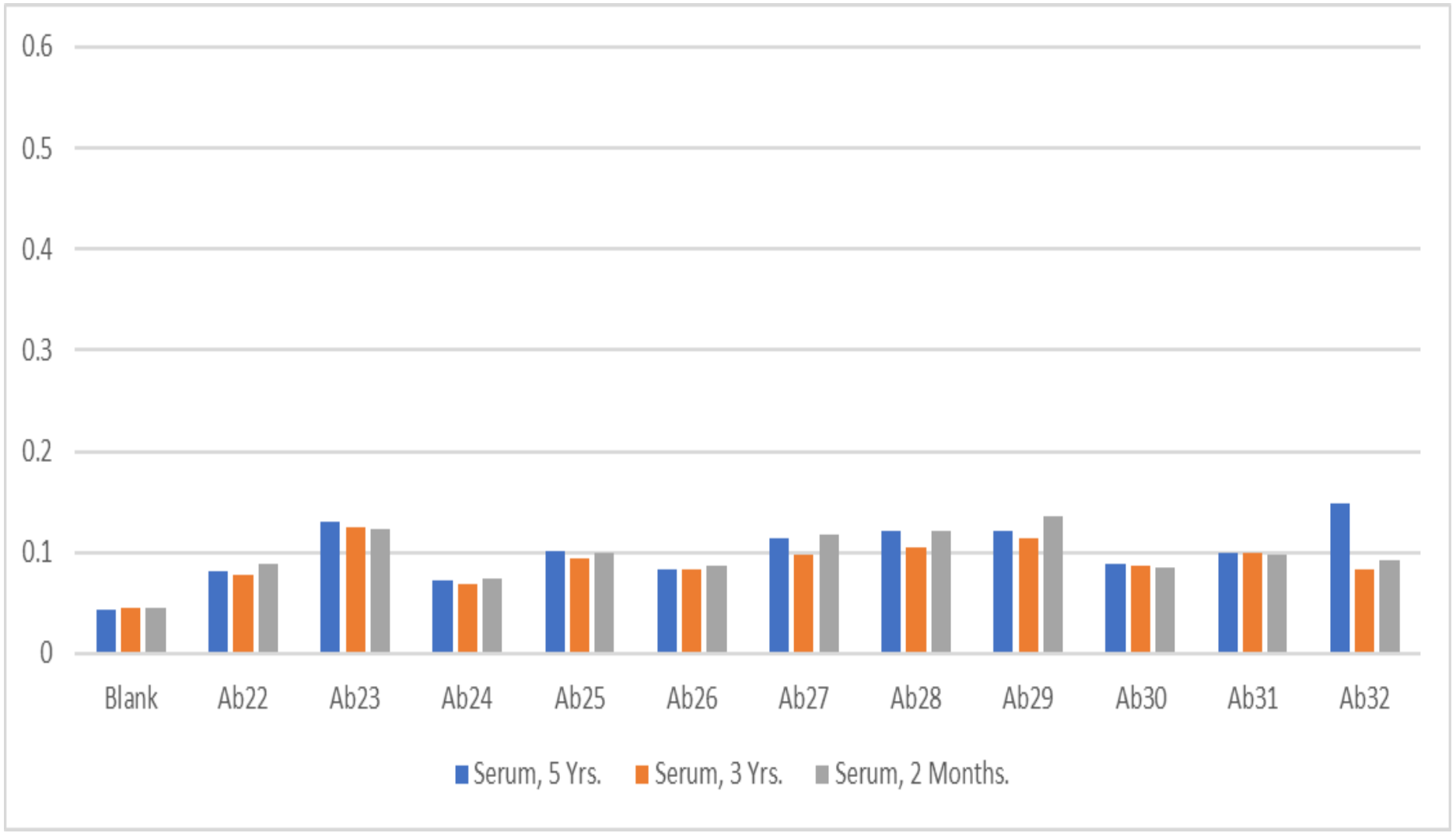
案例3. 反复冻融对小鼠单克隆抗体HOS的影响



案例4. 贮藏时间对纯化兔IgG HOS的影响



案例4. 储存时间对兔血清IgG HOS的影响



结论

1. 基础研究和药物开发中的关键试剂需要得到妥善管理。
2. PCA技术在检测人类，小鼠和兔源性关键抗体试剂的构象变化方面具有良好的灵敏度。
3. 抗血清和纯化抗体的长期和短期储存可以通过PCA ELISA评估其稳定性。
4. 作为最敏感的三维构像检测技术之一，PCA可以在很长的抗体使用时间内检测其潜在的构像变化从而达到对质量的控制。