

Minimizing False-Positive Results from Anti-CD20 Antibody Interference in Crossmatch Testing

Background

Anti-CD20 antibodies, such as rituximab and ofatumumab, are widely recognized for their efficacy in treating non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL) and a variety of autoimmune diseases. Additionally, rituximab is used as off-label medication to improve outcomes in ABO-incompatible kidney transplantation.¹ Despite its clinical success, rituximab poses challenges in crossmatch testing, as the presence of anti-CD20 antibodies in patient serum can lead to false-positive results in crossmatch assays, complicating the detection of donor-specific antibodies (DSA).² To address this issue, a novel CD20 blocking reagent, RituxiEx, is evaluated for its potential to minimize false-positive results and improve assay accuracy.

Methods

Complement-dependent cytotoxicity crossmatch (CDCXM) assays were performed using isolated human B cells from healthy donor lymphocytes. To evaluate the efficacy of RituxiEx in reducing false-positive results, cells were pre-incubated with RituxiEx or left untreated. After incubation, cells were exposed to purified anti-CD20 antibody. Rituximab and ofatumumab-induced CDC activity and false-positive outcomes were assessed by assigning a score to each sample based on the percentage of dead cells.

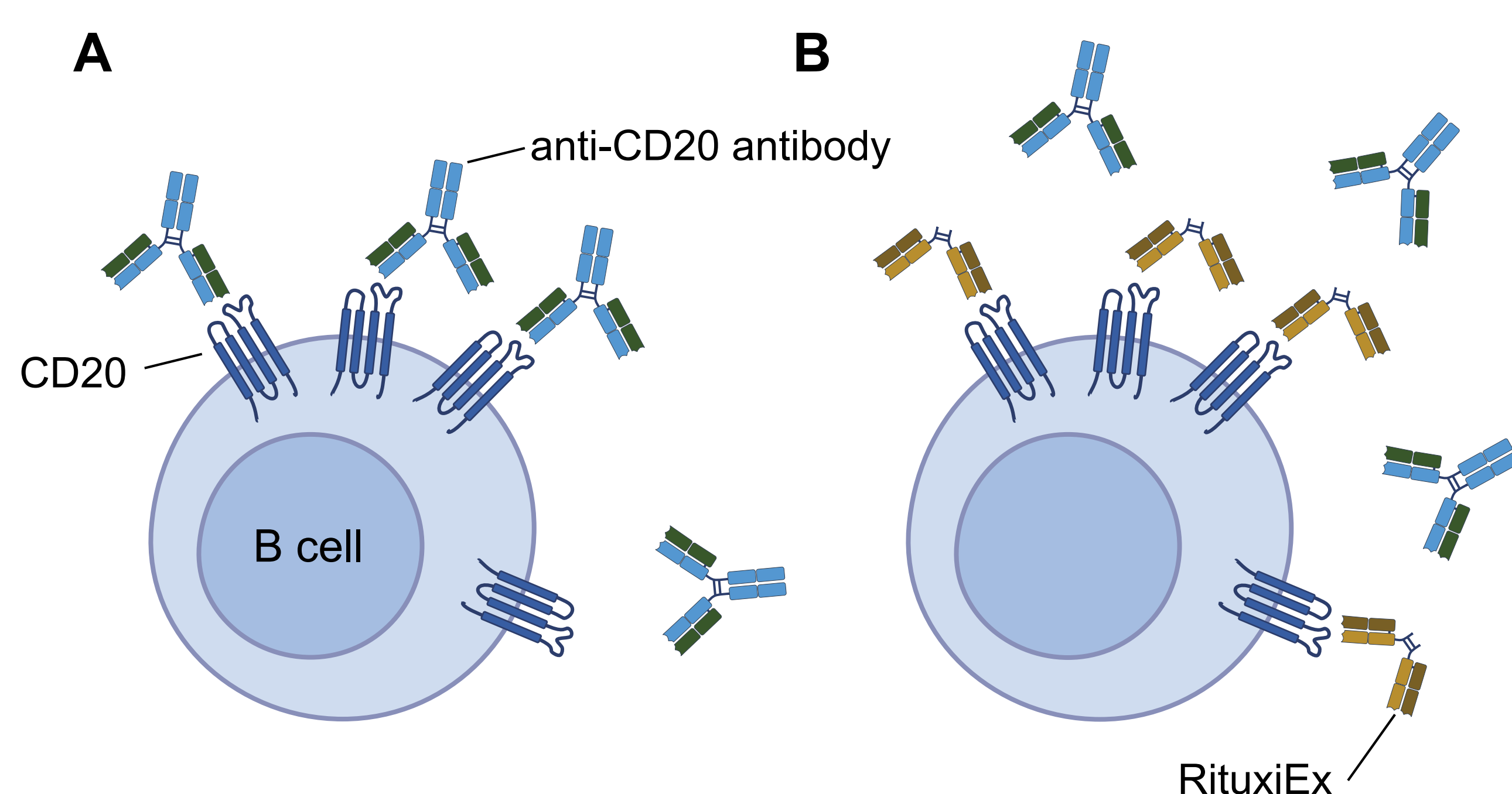


Figure 1. Principle of CD20 Masking with RituxiEx

(A) Anti-CD20 antibodies (e.g., rituximab) present in recipient serum can bind to CD20 on the B cell surface, activating CDC and leading to false-positive results in CDCXM assays. (B) Pre-treatment with RituxiEx masks CD20 on the B cell surface, preventing anti-CD20 antibodies from binding and reducing false-positive results, thereby improving assay accuracy.

Results

Pre-treatment of isolated B cells with RituxiEx effectively eliminates anti-CD20 antibody interference and rituximab-induced false positive results in crossmatch assays, with minimal effects on assay performance or cell viability (Figure 2). Additionally, ofatumumab-induced interference is markedly reduced (up to 250 µg/ml) or mitigated (up to 125 µg/ml) in RituxiEx-treated samples (Figure 3). Preliminary data further suggest a potential role in reducing rituximab-associated false-positive signals in flow cytometry crossmatch (FCXM) assays.

Conclusion

RituxiEx offers a novel and effective solution to improve diagnostic accuracy in transplantation crossmatch testing. By minimizing anti-CD20 antibody-induced false-positive results, the reagent enhances assay performance with minimal impact on routine laboratory workflows, representing a promising tool for clinical laboratories. However, further studies on patient samples are necessary to assess its viability for clinical implementation.

Conflict of Interest

PT and CS are employees of imusyn GmbH & Co. KG. CS is a designated inventor of the technology presented here.

Download poster at: https://www.imusyn.de/fileadmin/Dokumente/Poster_EFI2025_CD20.pdf

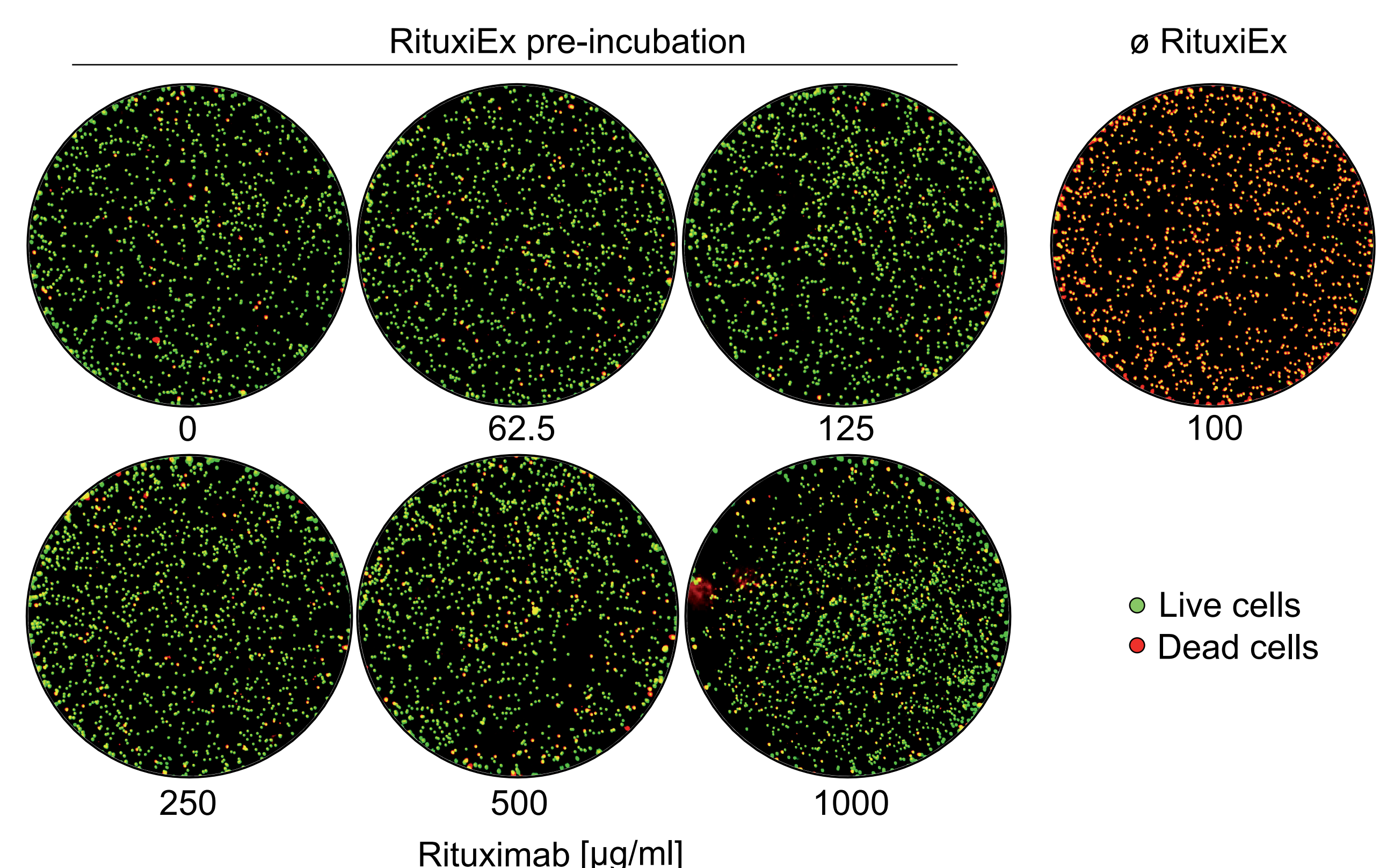


Figure 2. RituxiEx effectively prevents rituximab-induced false-positives in CDC assays at rituximab concentrations up to 1000 µg/ml. Isolated human B cells were pre-treated with RituxiEx and exposed to purified anti-CD20 antibody rituximab (62.5-1000 µg/ml) in PBS. FluoroQuench staining was performed to distinguish live (green) from dead (red) cells.

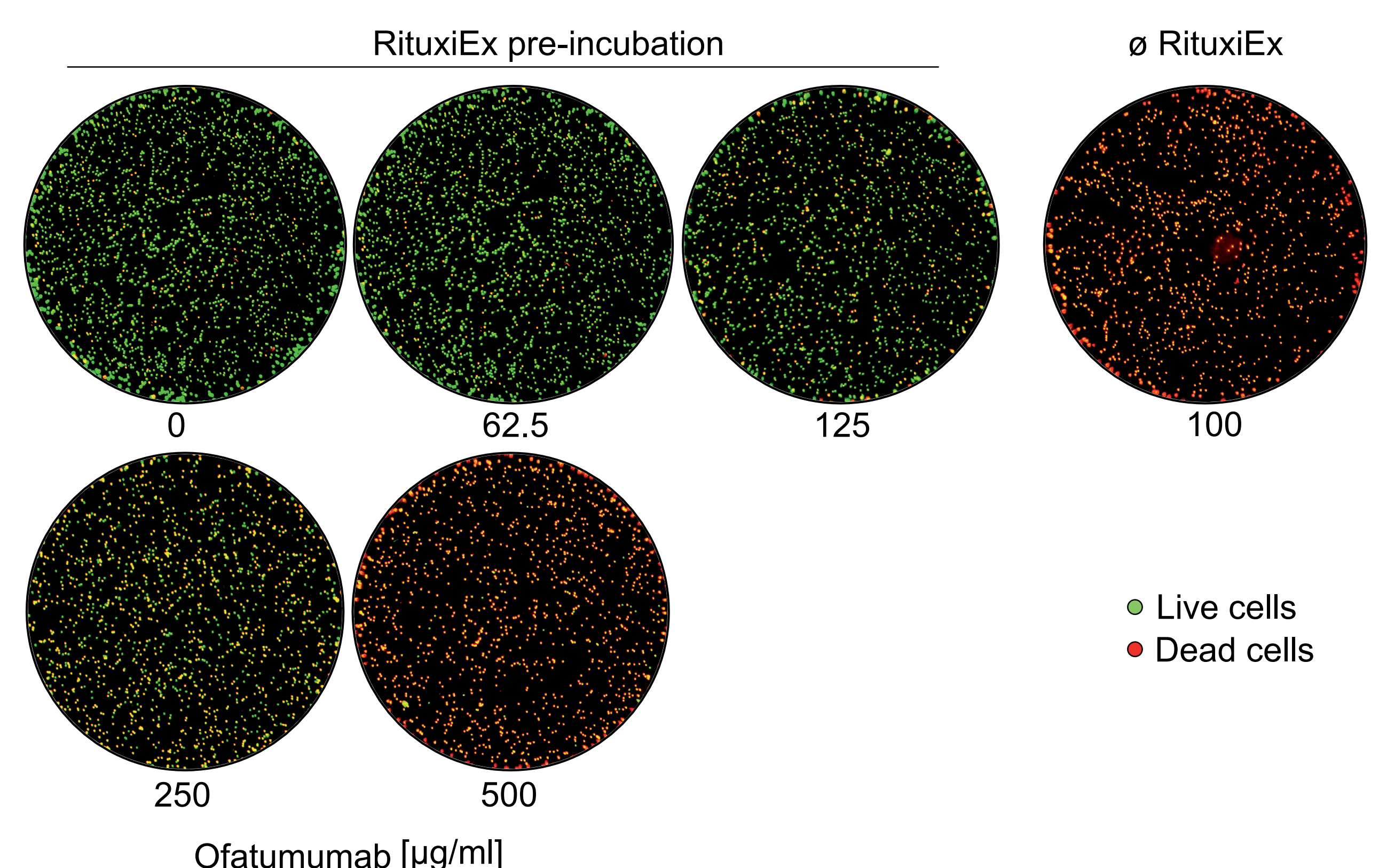


Figure 3. RituxiEx prevents ofatumumab-induced false-positives in CDC assays at ofatumumab concentrations up to 125 µg/ml and substantially reduces false-positives up to 250 µg/ml. Isolated human B cells were pre-treated with RituxiEx and exposed to purified anti-CD20 antibody ofatumumab (62.5-500 µg/ml) in PBS. FluoroQuench staining was performed to distinguish live (green) from dead (red) cells.

References

- 1 Genberg et al. (2007). Long-term results of ABO-incompatible kidney transplantation with antigen-specific immunoadsorption and rituximab. *Transplantation*, 84/12, S44
- 2 Milongo et al. (2015). Interference of therapeutic antibodies used in desensitization protocols on lymphocytotoxicity crossmatch results. *Transplant Immunology*, 32/3, 151-155

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