

CD36-Enhanced Red Blood Cells Improve the Detectability of anti-CD36 Antibodies in the Indirect Antiglobulin Test

BACKGROUND

CD36 is a blood group system that is normally expressed at very low levels on adult red blood cells (RBCs).¹ Therefore, the detection of anti-CD36 antibodies by the indirect antiglobulin test (IAT) is challenging, and **anti-CD36 antibodies may be overlooked often**. However, anti-CD36 antibodies have been reported to cause clinical complications,² and their role in pregnancy is not completely understood, given that fetal RBCs carry higher levels of CD36. Here, we describe a modification of the IAT that specifically increases sensitivity to anti-CD36 antibodies (**Figure 1**).

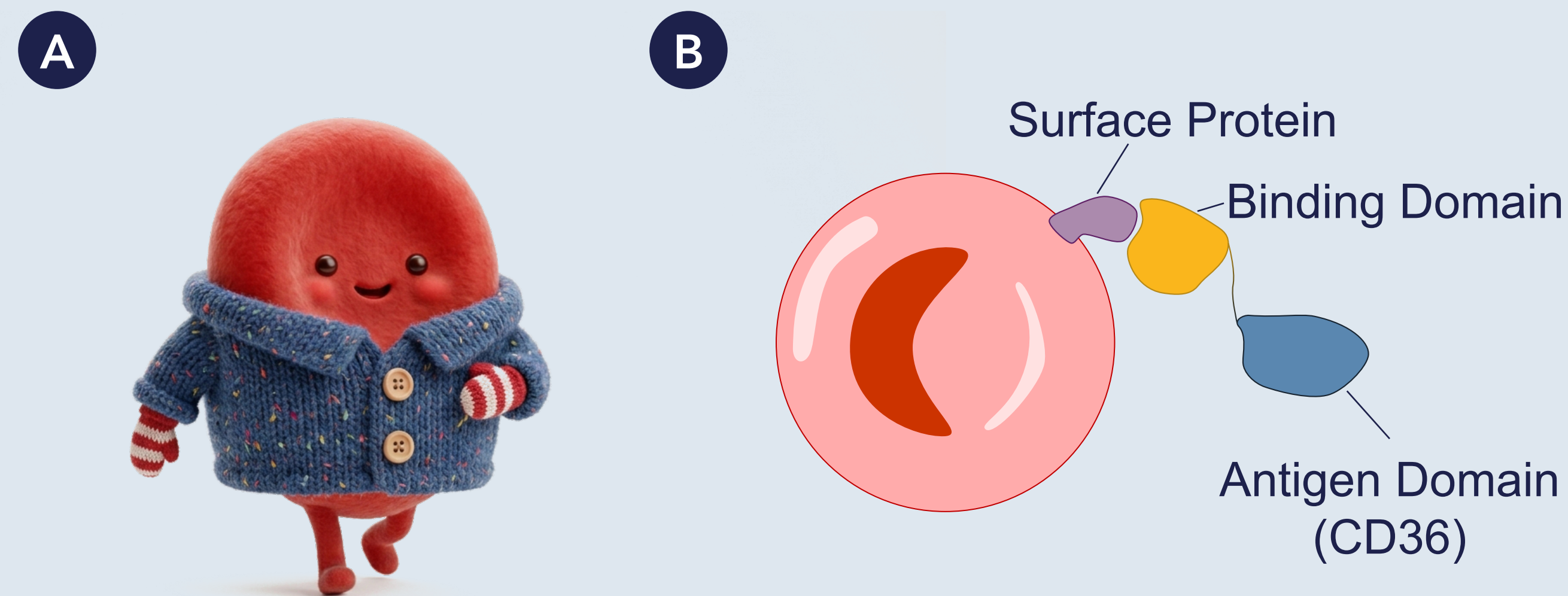


Figure 1. A: An RBC wearing a coat (AI generated). **B: Schematic drawing of the principle of eryCoat.** A fusion protein containing a binding domain (BD) for an RBC surface protein and an antigen domain, here CD36, binds to the surface of an RBC. The coated RBC can then be used for an agglutination test, for instance an indirect antiglobulin test (IAT).

METHODS

We designed a recombinant **fusion protein consisting of CD36 and an RBC-binding protein**, which was expressed in a mammalian cell culture system. RBCs were incubated with the cell culture supernatant and binding of the fusion protein was confirmed by flow cytometry. The treated cells were then used for IAT with anti-CD36 antisera, other antisera, and negative sera.

RESULTS

After optimization of the coating density (**Figure 2**), the **CD36-coated RBCs reacted strongly with anti-CD36 antisera**. This reaction could be inhibited by the addition of recombinant soluble CD36 (**Figure 3**).

CONCLUSION

eryCoat CD36 is a promising approach to filling a diagnostic gap in the detection of irregular antibodies. With this at hand, it should be easier to elucidate the clinical significance of CD36 as a blood group system.

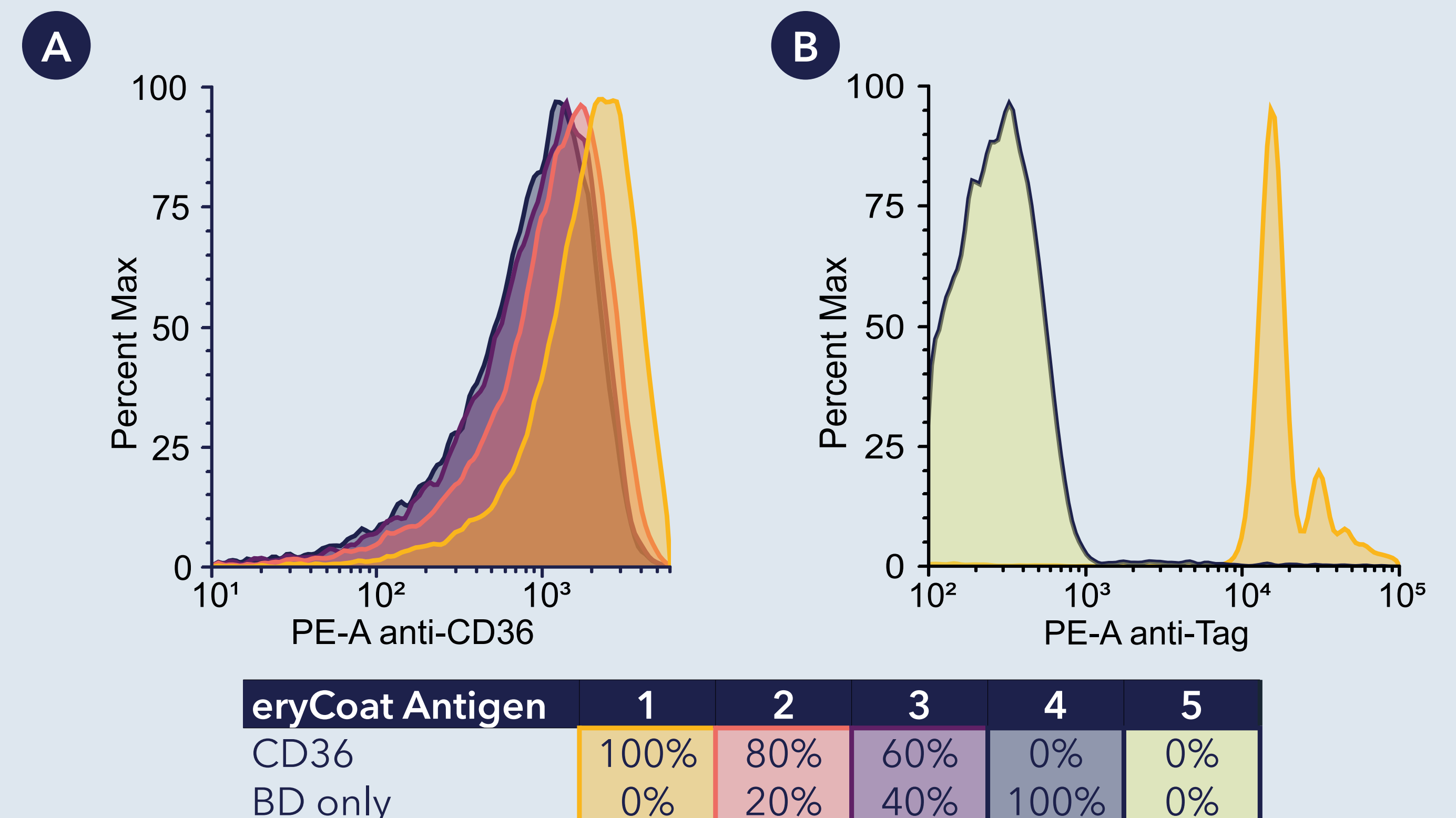


Figure 2. The CD36 fusion protein binds to RBCs. A: Flow cytometry analysis of a titration of a fusion protein with CD36 against the binding domain (BD) without antigen. Detection with an anti-CD36 antibody. **B:** Control staining with an anti-tag antibody.

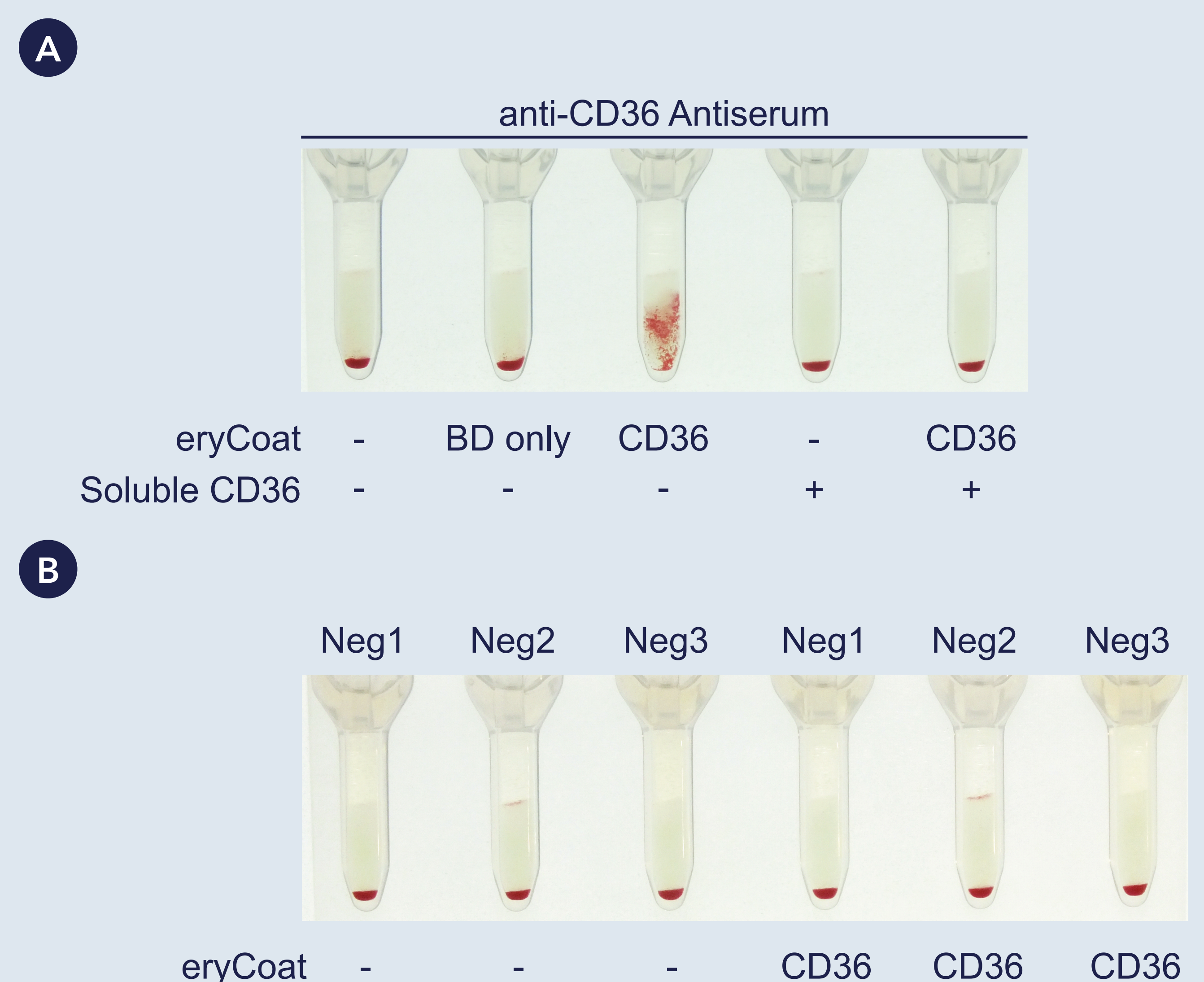


Figure 3. eryCoat CD36 enhances anti-CD36 reactions. IAT of uncoated and coated RBCs with **A:** an anti-CD36 antiserum and **B:** negative sera (Neg). Soluble recombinant CD36 (srCD36) was added to serum as indicated.

References

- Alattar AG, Storry JR, Olsson ML. Evidence that CD36 is expressed on red blood cells and constitutes a novel blood group system of clinical importance. Vox Sang. 2024; 119: 496-504.
- Okajima S, Cho K, Chiba H, Azuma H, Mochizuki T, Yamaguchi M, Sato S, Ikeda H, Yamada H, Minakami H, Ariga T, Kobayashi K. Two sibling cases of hydrops fetalis due to alloimmune anti-CD36 (Nak a) antibody. Thromb Haemost. 2006 Feb;95(2):267-71.

