

Prozone effect in autoimmunity: the case of islet cell antibodies

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Introduction

- Islet cells antibodies (ICA) are a marker for Type 1 Diabetes. It is tested by indirect immunofluorescence (IIF) on monkey pancreas (Fig 1). The recommended screening dilution for ICA by manufacturers is usually of 1:5 (Menarini) or 1:10 (EuroImmun).
- However, the sensitivity of this technique is known to be weak, leading to **false negative ICA results**.

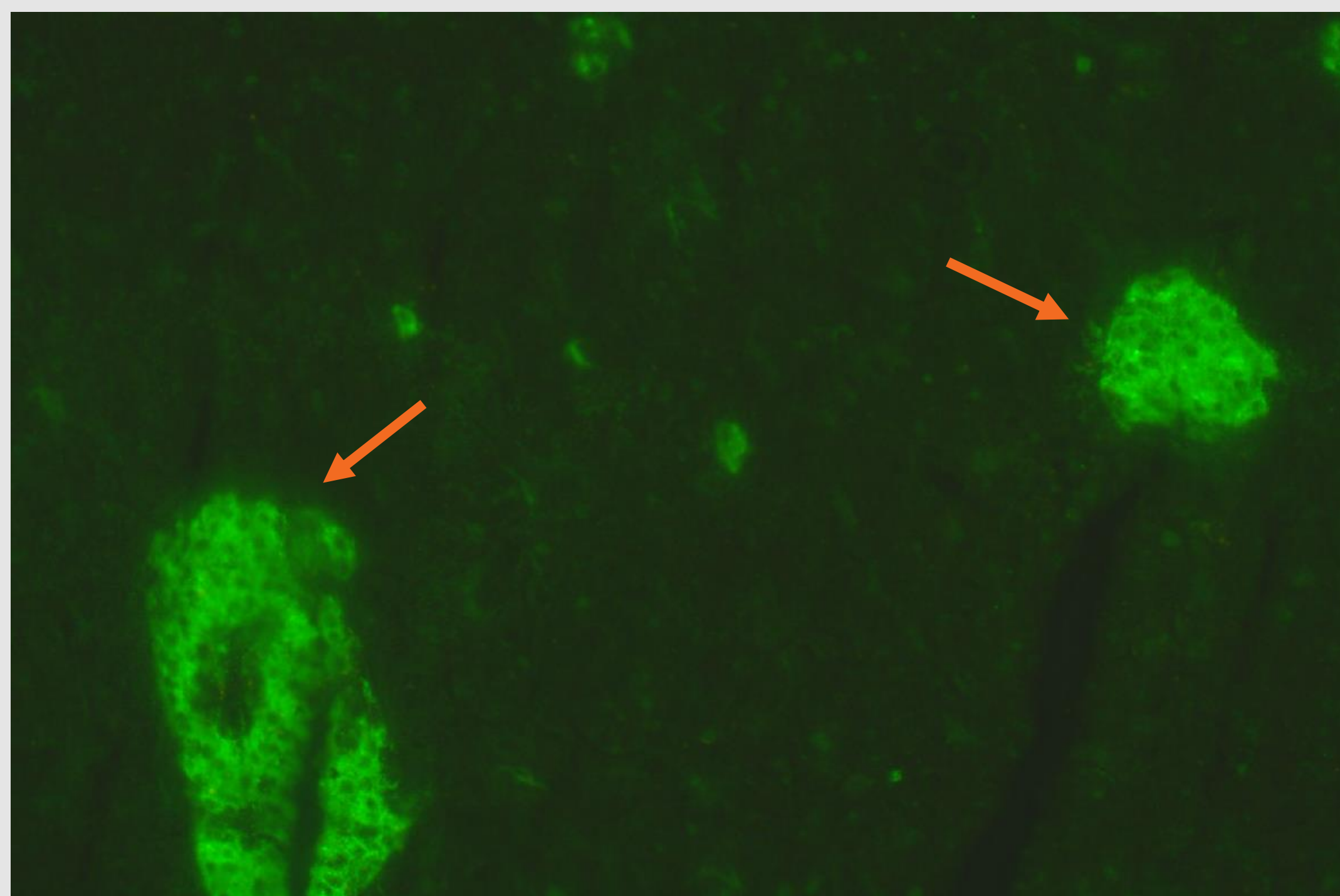


Figure 1: Monkey pancreas slide observed by IIF (40X) showing an ICA positive sample. The arrow indicates the Langherans islet displaying stronger fluorescence than the background)

Aim of the Study

- We sought to test different ICA IIF dilutions in order to improve ICA detection.
- In order to confirm these results, they were compared with anti-glutamic acid decarboxylase 65kd auto-antibodies (GADA) immunoassay. GADA is another test used for Type 1 Diabetes diagnosis, having higher sensitivity.

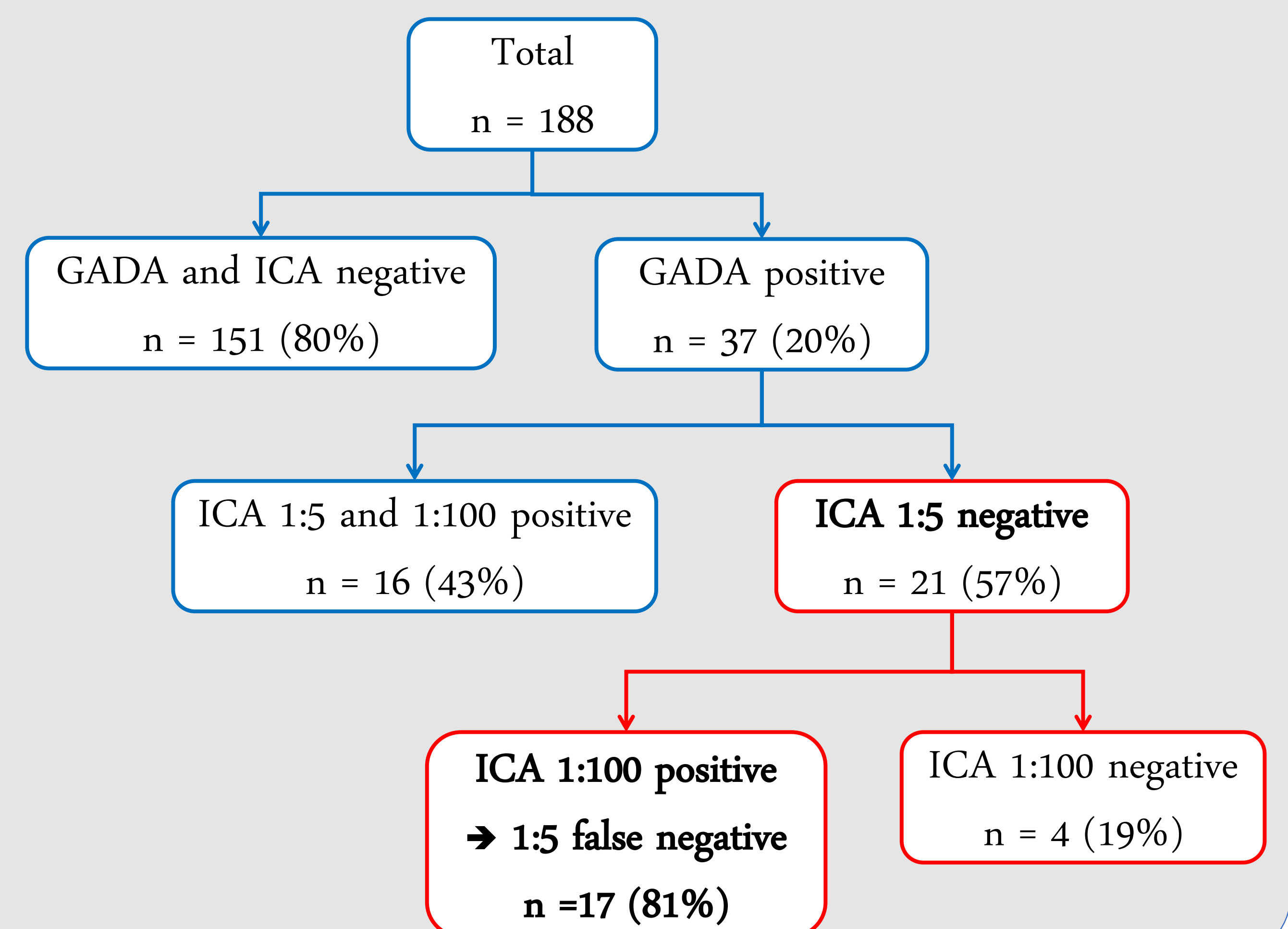
Material and Methods

- A total of 188 samples were prospectively collected between September 2019 and January 2021 at the CHU of Liège.
- All sera were tested for ICA by IIF (Menarini, ICAb Primate Pancreas) at 1:5 screening dilution according to the manufacturer recommendations. In addition, the samples were also tested at 1:100 dilutions to verify prozone (Hook) effect.
- GADA was measured by ELISA (IgG, Euroimmun) on Etimax (DiaSorin) to confirm the results.

Results

- The sera of 151 patients showed concordant negative results with GADA and the different ICA dilutions.
- Among those with GADA positives results, 16 (43%) samples had positive ICA at 1:5 dilution, but 21 (57%) were negative at this screening dilution recommended by manufacturers.
- When tested at higher dilutions, 17 (81%) of these negative sera became positive at 1:100 (Fig 2). This is due to a prozone effect at low dilution, which is avoided at higher dilutions (Fig 3), and was confirmed by anti-GADA positivity (1,2).
- Finally, four patients had positive GADA antibodies but no positive ICA at any dilution, which is in accordance with previously published data (3). Indeed, ICA titers decrease with age and tends to be negative in >80% of patients 7 to 11 years after the disease onset.

Figure 2: Distribution of results according to GADA and ICA at 1:5 and 1:100 testing



Conclusions

- We showed that the 1:5 screening dilution recommended by the manufacturer leads to high false negative rate. Prozone effect is a well-known drawback of assays using antibodies, however it is still underestimated.
- Hence, we recommend to use a 1:100 screening dilution for ICA IIF testing. This would allow to recover 52% of patients with false negative results.

References

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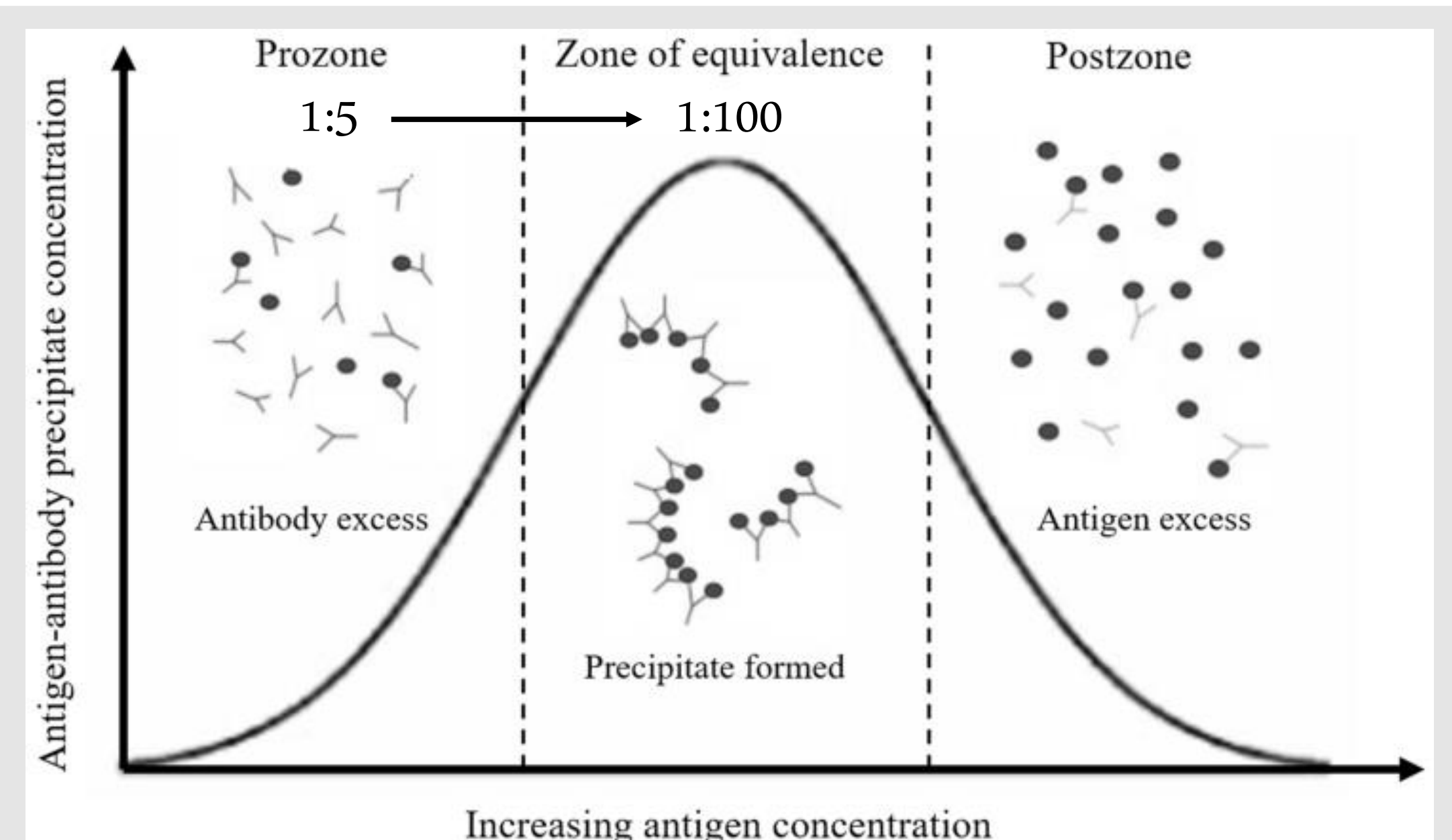


Figure 3: Prozone effect (Hook effect). When there is an excess of antibodies, the signal is weaker and lead to false negative results. Dilution allows to overcome this issue by reaching the equivalence level. Image from (4).