

Correlation of Tube and Automated Gel Isoagglutinin Titration Methods for Low-Titer Group O Whole Blood Selection



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Introduction

Low-titer group O whole blood (LTOWB) is increasingly being used in trauma resuscitation due to its physiological composition and logistical advantages. Control of anti-A and anti-B isoagglutinin titers is critical to minimize hemolytic transfusion reactions. Conventional tube titration remains widely used but is labor intensive and subject to variability. Automated gel-based methods may improve standardization; however, correlation with tube-based thresholds (1:100) is required to support clinical implementation without compromising donor availability.

Methods

This study evaluated the correlation between manual tube titration and automated neutral gel titration using the IH-500™ System for group O blood donors. A total of 159 plasma samples (119 random donors and 40 high-titer donors identified by the tube method) were included in the threshold development phases (Phases 1 and 2). In addition, 60 samples were prospectively tested in Phase 3 to validate the selected automated gel threshold. During Phase 1 (n = 60), tube titration was performed using serial twofold dilutions (1:25, 1:50, 1:100, and 1:200); automated gel testing used serial dilutions up to 1:2048 to establish candidate IH-500 thresholds. During Phase 2 (n = 99), a single tube dilution (1:100) was compared with automated serial dilutions up to 1:2,048 to determine the optimal gel cut-offs based on diagnostic performance. Phase 3 (n = 60) prospectively evaluated the selected gel thresholds using a single-point dilution against the conventional tube method at 1:100 (Table 1). Tube titration was performed by immediate-spin testing of each dilution against pooled A₁ and B reagent red blood cells in isotonic saline. Automated testing was performed on the IH-500 System using IH-Card ABO Neutral gel cards with A₁ and B reagent red blood cells (Figure 1). Tube titers were defined as the highest dilution showing visible agglutination, while automated gel titers were defined as the highest dilution with a reaction strength of ≥1+. Diagnostic performance was assessed by qualitative concordance and classification of samples as high- or low-titer using sensitivity, specificity, positive and negative predictive values, and the Youden index to identify the optimal automated gel thresholds.

Table 1. Study design and evaluation phases for selection and validation of the IH-500 automated gel threshold.

Phase	Tube Method	IH-500 System	Samples (n)	Objective
1 – Threshold exploration	Serial twofold dilutions (1:25–1:200)	Serial dilutions (up to 1:2048)	60	Evaluate the relationship between tube and automated gel titers
2 – Threshold optimization	Single dilution (1:100)	Serial dilutions (up to 1:2048)	99	Determine the optimal IH-500 System cut-off using diagnostic performance (sensitivity, specificity, Youden Index)
3 – Validation	Single dilution (1:100)	Selected single dilution	60	Validate the selected IH-500 System cut-offs in an independent cohort

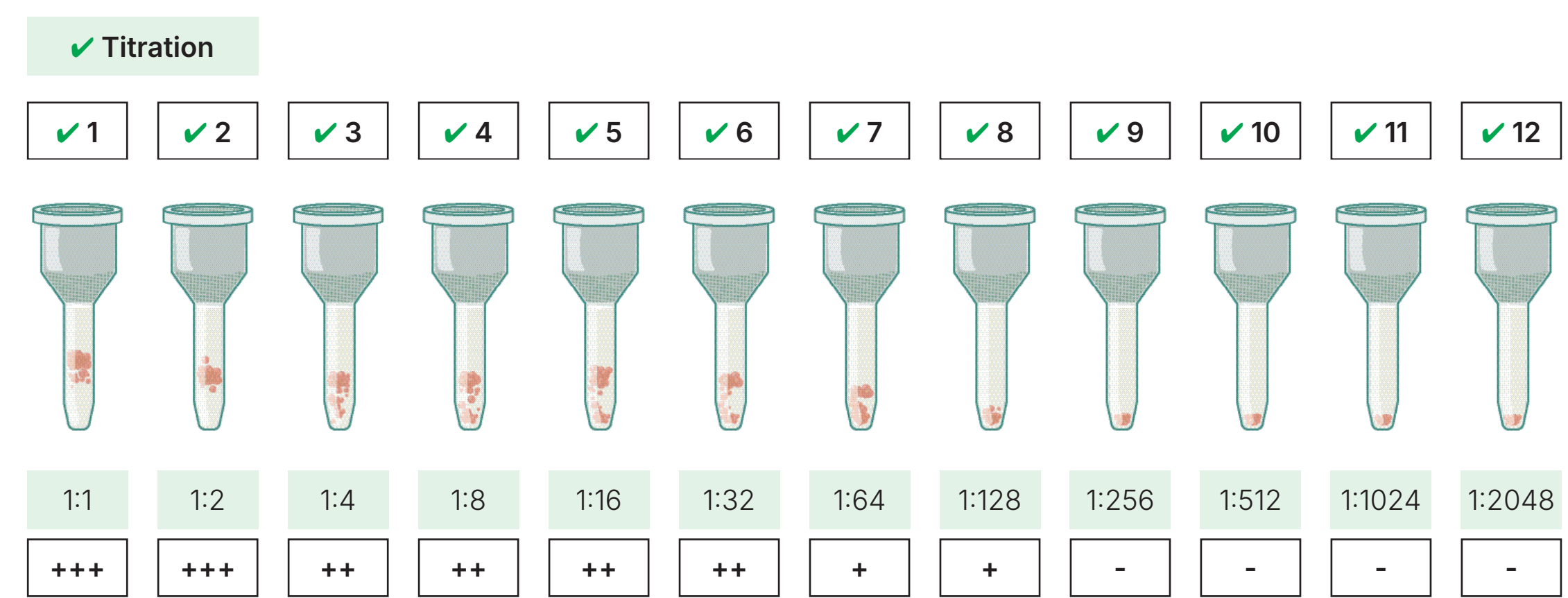


Fig. 1. Simulated examples of IH-Card titration results.



Fig. 2. Youden Index (Se+Sp-1) for candidate automated gel cut-offs.

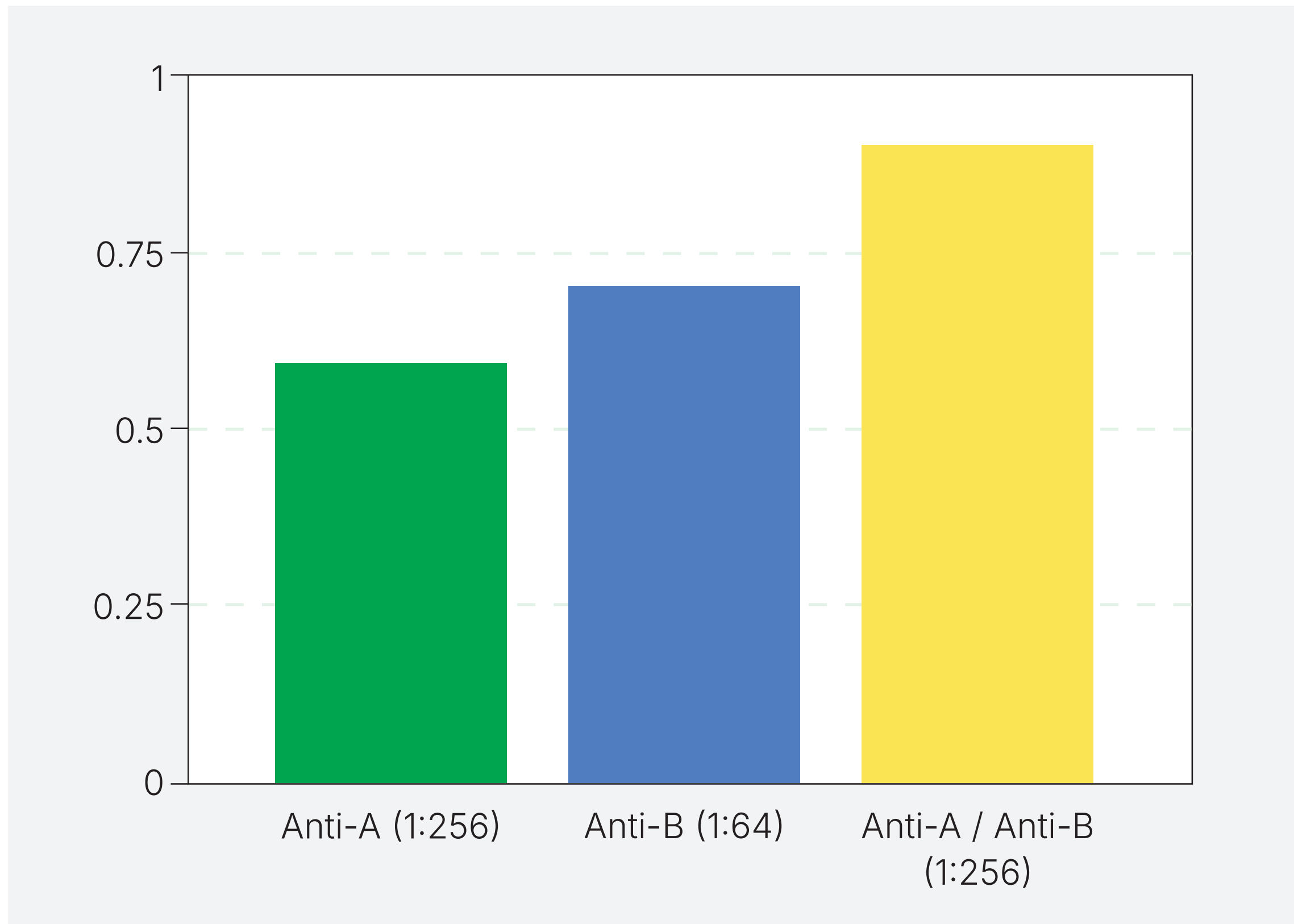


Fig. 3. Youden index for anti-A, anti-B, and the combined anti-A/anti-B algorithm in Phase 3.

Results

Data from Phases 1 and 2 were used to evaluate the relationship between tube titration at a 1:100 cut-off and automated gel titration using the IH-500 System. Candidate gel cut-offs were assessed using sensitivity, specificity, and the Youden index. The cut-offs providing the best agreement with the tube method were 1:256 for anti-A and 1:64 for anti-B. The combined thresholds (anti-A ≥ 1:256 and anti-B ≥ 1:64) achieved a sensitivity of 95%, specificity of 75%, and Youden index of 0.70, with a PPV of 56% and an NPV of 98% (Figure 2). Phase 3 evaluated these thresholds in 60 independent plasma samples using single-point dilutions (anti-A: 1:256; anti-B: 1:64), compared with the reference tube method at 1:100. The anti-A threshold showed lower performance than in Phases 1 and 2 (Youden index: 0.59), whereas performance for anti-B remained comparable (Youden index: 0.70). When combined, the two thresholds achieved a Youden index of 0.90, with a PPV of 100% and an NPV of 95% (Figure 3). Although the anti-A threshold may warrant further optimization, the performance of the combined algorithm was not negatively affected. The use of single-point dilutions in place of serial twofold titrations reduces testing steps and is expected to reduce hands-on time and reagent consumption while maintaining high diagnostic performance relative to the reference tube method.

Conclusion

Automated gel isoagglutinin titration using the IH-500 System demonstrated very good agreement with conventional manual tube titration when method-specific cut-offs (anti-A ≥ 1:256 and anti-B ≥ 1:64) were applied. The proposed single-point automated gel approach provides a practical alternative to serial tube titration, improving workflow efficiency, standardization, and reproducibility while maintaining high diagnostic performance. Validation in a larger, independent donor cohort is warranted to confirm these findings and support implementation for LTOWB donor screening.

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