Dear Editor,

In Korea, many blood banks test ABO blood groups using automated analyzers [1]. This reduces costs and increases the work efficiency compared with manual methods [2]. The IH-1000 (Bio-Rad Laboratories, Hercules, CA, USA) automated analyzer used in Asan Medical Center, Seoul, Korea, functions based on the column agglutination principle. Results obtained using the IH-1000 analyzer reportedly show a good correlation with those obtained using the manual tube method [3]. ABO subgroups are difficult to determine using an automated analyzer. Few studies have investigated this, and most were not conducted in a clinical environment [1, 3]. We compared final blood group determination by manual retesting with those determined using the IH-1000 analyzer using real-world data. Based on the analysis results, we provide information to assist clinical interpretation of hemagglutination grading when using an automated analyzer. The Institutional Review Board of Asan Medical Center approved this study (2020-0392).

In total, 209,668 samples tested for ABO blood groups between September 2017 and January 2020 were retrospectively investigated. We compared the results determined using the IH-1000 analyzer and the final reading results (i.e., those obtained using the IH-1000 analyzer only or those confirmed by manual retesting) (Table 1). A final blood group determination by manual retesting was required in the following cases: 1) new patients with no prior blood group data, 2) a grade ≤+3 in forward typing, 3) a grade ≤+2 in reverse typing, and 4) analyzer results other than positive and negative reactions, and 5) no reading output from the analyzer for any other reason.

When comparing the IH-1000 results with those of the manual testing for the forward typing of antigens A and B in 24,629 samples, 99.9% of the samples showing a reaction grade ≥3+ in the IH-1000 analyzer showed grade 4+ in the manual testing. Moreover, with the same 4+ grade in the manual test, the reaction grades for antigens A and B differed in the IH-1000 analyzer. The samples determined to have grade 3+ by the IH-1000 analyzer were substantially higher for antigen B (59.2%) than for antigen A (24.0%).

The 3+ samples were comprehensively analyzed (Table 2). Most samples showing a grade ≥3+ in both the A and B blood groups were classified as blood group AB in the manual retesting. AB subgroup results were obtained in only a few samples. Among the AB subgroup samples, anti-B antibody was detected by reverse typing in 14 out of 15 A_2B_3 samples, and anti-A1 an-

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tibody was detected in 1 out of 2 A\textsubscript{2}B samples. When antigen A was grade 3+ and antigen B was negative, the final interpretation was that of blood group A. When antigen A was negative and antigen B was grade 3+, most samples were classified as blood group B, and a few were subgrouped as A\textsubscript{2}B\textsubscript{3} or B\textsubscript{3}. We identified 168 cases with an ABO subgroup (0.08%), which is similar to the proportion (0.05%) reported in a previous study of Korean blood donors [4]. The following blood subgroups were determined: 38 A\textsubscript{2}B\textsubscript{3}, 28 AB\textsubscript{3}, 25 ABw, 24 B\textsubscript{3}, 16 Aw, 9 Bw, 7 AwB, 6 A\textsubscript{2}B, 6 A\textsubscript{2}, 4 A\textsubscript{2}w, 2 A\textsubscript{2}Bw, 1 A\textsubscript{2}B, 1 AwBw, and 1 Bx.

Table 2. Analysis of samples with a reaction grade 3+ in the IH-1000 analyzer

| A/B antigen | Automation | Manual | N | %  | Final interpretation |
|-------------|------------|--------|---|----|---------------------|
| 4+/3+       | 4+/4+      | 2,640  | 99.36 | AB |
| 4+/3+       | 2          | 0.08   | AB (1), ABw (1) |
| 4+/2+       | 15         | 0.56   | A-B\textsubscript{3} |
| 3+/4+       | 4+/4+      | 613    | 100  | AB (611), A-B\textsubscript{2} |
| 3+/3+       | 4+/4+      | 259    | 99.62 | AB (258), AwB (1) |
| 2+/4+       | 1          | 0.38   | AwB |
| 3/-         | 4/-        | 2,470  | 100  | A |
| -/3+        | -/-        | 4,876  | 99.96 | B (4,875), B\textsubscript{1} |
| ±/4+        | 1          | 0.02   | AwB |
| -/2+        | 1          | 0.02   | B\textsubscript{3} |

| A/B antigen | Automation | Manual | N | %  | Final interpretation |
|-------------|------------|--------|---|----|---------------------|
| 4+/3+       | 4+/4+      | 2,640  | 99.36 | AB |
| 4+/3+       | 2          | 0.08   | AB (1), ABw (1) |
| 4+/2+       | 15         | 0.56   | A-B\textsubscript{3} |
| 3+/4+       | 4+/4+      | 613    | 100  | AB (611), A-B\textsubscript{2} |
| 3+/3+       | 4+/4+      | 259    | 99.62 | AB (258), AwB (1) |
| 2+/4+       | 1          | 0.38   | AwB |
| 3/-         | 4/-        | 2,470  | 100  | A |
| -/3+        | -/-        | 4,876  | 99.96 | B (4,875), B\textsubscript{1} |
| ±/4+        | 1          | 0.02   | AwB |
| -/2+        | 1          | 0.02   | B\textsubscript{3} |

In conclusion, a 3+ reaction grade determined using the IH-1000 automated analyzer will generally be assessed as a 4+ grade using a manual method. The proportion of samples with grade 3+ was higher for antigen B than for antigen A. A blood group reading generated by the IH-1000 analyzer can safely be used for transfusion purposes without additional testing. When the antigen reading on this instrument shows a reaction grade 3+, the patient may have an ABO subgroup. Manual retesting and reverse typing are safe choices for blood group determination.

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AUTHOR CONTRIBUTIONS
Park B summarized the data and wrote the manuscript. Kim JS contributed to the data collection. Youk HJ, Chung Y, and Kim H critically revised the manuscript and supported the study. Hwang SH and Oh HB supported and supervised the study. Ko DH designed and supervised the study.

CONFLICTS OF INTEREST
None declared.

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