On the high probability that a perceived lack of value of obtaining a p value will be detrimental to patient care!

We are privileged to be invited by the Editor to comment on the article by Kanter et al.[1] in this issue of TRANSFUSION purporting a lack of value in obtaining a p value of <0.05 (95% confidence level) when performing antibody identification studies. We are especially concerned that the vast majority of technologists performing these studies will now reject the "3+/3– rule" promulgated by their mentors. This can only have a negative impact on patient care.

Kanter et al. claim that Judd is wrong in stating, "[A] p value of 0.05... means that an identical set of reactions due to an antibody other than D could be obtained by chance once in 20 similar studies."[2(p211)] They consider Menitove[3] correct in stating that Fisher's exact test is actually a test for statistical independence between test results and the presence or absence of a specific antigen. However, in accepting the null hypothesis, one is stating that the probability of obtaining a positive test with antigen-positive red cells (RBCs) equals the probability of obtaining a positive test with antigen-negative RBCs. In rejecting the null hypothesis, one is stating that the reactions with antigen-positive RBCs and antigen-negative RBCs have different underlying probabilities. In this sense, the statement of Menitove[3] is correct. However, the p value, or the level of significance, is also "the probability of rejecting a true null hypothesis." In this sense, Judd is also correct.[2] If the null hypothesis is indeed true, then p is the probability of obtaining the observed results by chance.

While one can argue the "science" of statistics, it is imperative not to lose sight of the fact that application of the 3+/3– rule is but one of several measures used to enhance the quality—and hence, the accuracy—of antibody identification. In addition to validating negative antiglobulin tests with IgG-coated RBCs, other measures (notable exceptions aside) include confirming 1) that the test phase of reactivity is consistent with the concluded specificity; 2) that the antigen to which the serum is deemed to contain alloantibody is absent from the autologous RBCs; 3) that the concluded specificity is consistent with results of the initial antibody detection tests; and 4) that units lacking antigens to which the serum is deemed to contain antibody are crossmatch-compatible. Further, most workers utilize reagent RBC panels of 8 to 11 samples, and the confidence level obtained is often far greater than 95 percent. However, the 3+/3– rule do become important when multiple antibodies are present and the numbers of positive and negative samples are limited.

In calculations of the probability that a particular antibody is present, the sensitivity and specificity of the test method employed also must be taken into consideration. The probability (call it p) that a positive test with an antigen-positive RBC sample is a true positive (i.e., is due to the presence of specific antibody) is equal to the predictive value of a positive test. The probability (call it q) that a negative test is a true negative is equal to the specificity of the method used. Assuming that individual serologic tests are independent, the probability that the decision rule will be met is given by the formula $r = (1-(1-p)^n)\,(1-(1-q)^n)$, where n is the number of antigen-positive and antigen-negative RBC samples used (e.g., 2 or 3). The rate of identification failures per 1000 samples is given by 1000(1–r). Table 1 gives the likelihood that an antibody is present given either a 3+/3– rule (criterion used by US workers) or a 2+/2– rule (UK requirement), assuming different levels of test sensitivity and specificity. It is evident that using 2 antigen-positive and 2 antigen-negative RBC samples significantly reduce the confidence one can have in antibody identification even with excellent test performance.

We have seen the consequences of too many misidentified antibodies not to come to believe that it would be less than prudent to encourage workers to forget the 3+/3– rule or any of the other quality assurance measures that are part of good laboratory practice.

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TABLE 1. Influence of test sensitivity and specificity on probability of correct identification

Sensitivity	Specificity	3+/3– rule	2+/2– rule
0.95	0.95	0.954 (46)*	0.858 (142)
0.75†	0.95	0.929 (71)	0.826 (174)
0.95	0.75‡	0.591 (409)	0.429 (571)

* Probability of correct identification and expected number of incorrect antibody identifications in parentheses, including failure to identify an antibody that is present, per 1000 studies.
† High number of false negatives as in improper incubation conditions.
‡ High number of false positives as seen with interference from additional (unidentified) antibodies, autoantibodies, and false reactivity.

REFERENCES

1. Kanter MH, Poole G, Garratty G. Misinterpretation and misapplication of p values in antibody identification: the lack of value of a p value. Transfusion 1997;37:816–22.
2. Judd WJ. Methods in immunohematology. 2nd ed. Durham, NC: Montgomery Scientific Publications, 1994.
3. Menitove JE. The Hardy-Weinberg principle: selection of compatible blood based on mathematic principles. In: Fridey JL, Kasprisin CA, Chambers LA, Rudmann SV, eds. Numbers for blood bankers. Bethesda: American Association of Blood Banks, 1995:1-11.