Carrier Detection in Hemophilia A: A Cooperative International Study.
II. The Efficacy of a Universal Discriminant

By Philip P. Green, Piero Mannuccio Mannucci, Ernest Briët, Rolf Ljung, Carol K. Kasper, E.M. Essien, Juan Chediak, Charles R. Rizza, and John B. Graham

Factor VIII (F.VIII) and von Willebrand factor (VWF):Ag data collected by eight laboratories on a total of 336 obligatory carriers of hemophilia A and 137 normal women were used to answer several questions concerning the construction of linear discriminants for carrier detection. It was found: (a) that a “universal” linear discriminant can be constructed which is suitable for use in all laboratories and is nearly as effective as laboratory-specific discriminants; (b) that inclusion of age and ABO blood type data improved the efficacy of these discriminants; (c) that substitution of alternative assays for F.VIII and VWF:Ag did not generally improve the efficacy of the discriminants over that obtained using the bioassay for F.VIII:C and Laurell's immunosassay for VWF:Ag; (d) that linear discriminants were far more effective than discriminants based on the F.VIII:C/VWF:Ag ratio. A step-wise procedure is given which any laboratory may follow in using the universal discriminant for carrier detection.

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LINEAR discriminants which incorporate data on (Factor VIII) F.VIII:C and (von Willebrand factor) VWF:Ag levels are well established tools for estimating the probability of carriership of hemophilia A, and together with newer but less widely applicable recombinant DNA techniques they are likely to remain important in genetic counseling for many years. The exact manner in which the assay data are combined to give the discriminant value depends on a mathematical expression whose exact form may vary, but which is to be chosen so that the populations of carrier and of normal women have as little overlap of their discriminant values as possible. Elston and colleagues suggested that interlaboratory differences in assay methods and/or reference populations make it necessary for each laboratory to construct its own discriminant. However, some laboratories may not have access to a large enough carrier population to permit the calculation of an accurate discriminant.

In the present study, data collected in a multicenter international study involving eight laboratories have been used to examine in detail the feasibility of a common discriminant for use in multiple laboratories. The common thread linking the studies of the different laboratories was a single lyophilized plasma standard. The form that such a “universal” discriminant should take to optimize discrimination was also examined, since no clear consensus has emerged from previous studies. The specific issues considered here include: the importance of correcting for age and for ABO blood group, both of which have appreciable effects on F.VIII:C and VWF:Ag levels in both normal subjects and carriers; the effect of correcting for differences in mean values between laboratories; whether assays other than the customary bioassay for F.VIII:C and Laurell’s electroimmunosassay for VWF:Ag can significantly improve discrimination; and the relative accuracy of the widely used ratio method for discrimination.

MATERIALS AND METHODS

The motivation and the design of the study are described more fully in our companion article. In brief, eight different laboratories assayed a total of 336 obligatory carriers and 137 normal women for F.VIII:C and VWF:Ag levels, each laboratory using a lyophilized plasma standard provided by the Oxford Haemophilia Centre, but otherwise using its own “in-house” methods. No subjects were pregnant, and only five were using oral contraceptives. Because no differences were detected between “maternal” and “paternal” carriers, their data were pooled with those of “sporadic” obligatory carriers for the purposes of computing the discriminants and determining misclassification rates and average odds.

Statistical methods for computing covariate-adjusted discriminants were for the most part the same as previously described. The F.VIII:C and VWF:Ag values were logarithmically transformed to reduce their skewness and that of the discriminants computed from them. Initial regression studies indicated that a quadratic term was necessary to correct fully for age effects on both F.VIII:C and VWF:Ag, and that although the mean levels of the laboratories differed, the laboratories did not differ in the effect which age had on their carrier populations. Furthermore, ABO blood type was found to have a significant effect on F.VIII:C and especially on VWF:Ag. Linear age-adjusted discriminants were computed for each laboratory and for the entire (pooled) sample, according to the method described by Elston and co-workers. To assess the effect on the discriminant of individuals with extreme ages, discriminants were also computed using only individuals with ages between 18 and 50. For the laboratories having data on ABO blood type (four of eight), discriminants were constructed which adjust for this variable in addition to age; the procedure for doing this is identical with that

--Following recent nomenclature conventions, F.VIII:C will be used to designate the clotting activity which is missing in severe hemophilia A measured by bioassay, and F.VIII:Ag (previously FVIII C:Ag) to designate it when determined immunologically. VWF:Ag (previously VIII R:Ag) refers to the antigen that is absent in severe von Willebrand’s disease.

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described for age-adjusted discriminants by Elston and co-workers, except that in each regression a class variable with two classes (types O and Non-O) is included in addition to the linear and quadratic age terms. For the three laboratories performing alternative assays for F.VIII:C and VWF:Ag in addition to the customary ones, discriminants were constructed using these alternative data. Finally, discriminants were computed based on the F.VIII:C/VWF:Ag ratio. Quadratic discriminants, which have occasionally been used in other laboratories, were not computed on this data set, because they are quite sensitive to how the data are transformed and have other undesirable properties.

For each type of discriminant, two measures of efficacy were computed, the “average odds” and the “misclassification rate.” The odds for carriership were computed for each carrier based on the value of her discriminant. Odds greater than 100:1 were “trimmed” to 100:1, to make the summary measures more robust to outliers, although some calculations were also done using untrimmed odds to determine how individuals with very high odds might affect the results. The (geometric) average of the odds for carriership was then computed for all carriers in the sample. High average odds indicate that, on average, the discriminant assigns a high probability of carriership to carriers. As is pointed out in the WHO Memorandum on carrier detection, a t test for comparing the efficacy of two discriminants may be performed using the logarithm of the ratio of their average odds. The other method of evaluation, “misclassification rate” for carriers, is simply the percentage of misclassified carriers in the sample, in which a carrier is arbitrarily considered misclassified if her odds for carriership are <1:1. A similar procedure was used to compute average odds and misclassification rates for the normal controls, misclassified normal controls being those with odds for carriership >1:1.

From the viewpoint of genetic counseling, average odds are clearly a more sensitive and useful measure of the behavior of a discriminant than is the crude misclassification rate since they take into account the magnitude of the probabilities assigned to individuals, rather than the relations of individual values to an arbitrary “cut-off” point. The misclassification rate is useful, however, in assessing the behavior of discriminants for individuals “on the borderline,” i.e., having odds of <1:1. Accordingly, we have included both summary measures in Tables I through 5.

Ideally, a sample used to estimate a discriminant should not contain the subjects who are going to be used to judge its efficacy, since, if it does, the resulting estimate of the misclassification rate (or average odds) will be biased. Consequently, the following method was used to obtain an unbiased estimate of the accuracy of a pooled, or “universal,” discriminant. For each laboratory, a discriminant was computed using data pooled from the other seven laboratories. This discriminant was then used to compute odds for carriership of, and to classify, each individual from the given laboratory’s sample, and the average odds and misclassification rates for that laboratory.

Total misclassification rates and average odds were produced by taking a weighted average over all laboratories. This method is analogous to the “leaving one out” (or “jack-knife”) method proposed by Lachenbruch but is computationally far easier to implement in the case of a covariate-adjusted discriminant. Furthermore, it more directly addresses the issue of interest here; namely, whether a discriminant computed from data produced in one group of laboratories can validly be applied to that of other laboratories. The average odds and misclassification rates reported below for the “universal” discriminant were obtained by this method and are, therefore, unbiased. The values reported for the “laboratory-specific” discriminants, however, were computed using the sample from which the discriminant was generated and are therefore somewhat biased.

Sample sizes varied, because the data base is not homogeneous, lab by lab. All eight laboratories provided data on carriers whereas only five provided a normal cohort. Four laboratories provided ABO blood group data, but only three of these also provided a normal cohort. For each discriminant, the average odds are greater for carriers than for normal subjects, and the rate of misclassification is greater for carriers than for normal subjects; both tendencies are due to the fact, itself a consequence of “lyonization,” that the distribution of carriers’ values for the discriminant has a higher variance than the distribution of normals’ values.

Computer programs to compute covariate adjusted discriminants, average odds, and misclassification rates, were written in SAS language and run on an IBM 3081 computer.

RESULTS

To investigate the general feasibility of a “universal” discriminant, we examined what appeared to be the pertinent variables from analyses described in our companion article. These were age, ABO blood group, and interlaboratory variation in mean levels. First, the efficacies of laboratory-specific discriminants computed with and without corrections for age and ABO blood group were compared. These were initially done using “untrimmed” odds and then repeated using the “trimmed” odds. The results with the trimmed odds are shown in Table 1.

The analyses using “untrimmed” odds (not shown) indicated that correction for age and ABO blood group significantly improved the average odds. This improvement seems to have come primarily from individuals having relatively large odds, since when “trimmed” odds are used the improvement vanishes entirely for age correction alone and is relatively small for the combined age and ABO blood group correction, and since the corrections do not improve misclassification rates (Table 1, lines 1 through 3). The (unbiased) estimates obtained with the universal discriminants using “trimmed” odds (cf. lines 4 and 5) show a similar pattern.

To determine whether individuals with extreme ages were having large effects on the discriminants, we recomputed the laboratory-specific discriminants using only individuals between the ages of 18 and 50 years (lines 6 through 8). The samples were smaller, and the results were inconsistent; with respect to the age and ABO adjusted discriminant, the average odds and the misclassification rates improved for normals when the truncated age range was used, but they worsened for carriers (line 8 v line 3).

We then investigated (Table 2) the effect of interlaboratory variation, since there are significant differences in mean levels of F.VIII:C and VWF:Ag between laboratories. Not surprisingly, the laboratory-specific discriminant is appreciably more efficient than the standard “universal” discriminant when adjusted for age and ABO only (cf lines 1 and 2). This could be due in part to the intrinsic bias in average odds and misclassification rates of laboratory-specific discriminants, but it probably also reflects local differences in assay methods and/or carrier populations.

To see whether correction for interlaboratory variation in mean levels would improve the efficacy of the “universal” discriminant, two adjustments were tried. First, the mean levels of the log-transformed F.VIII:C and VWF:Ag values for the carriers in each laboratory were computed and were subtracted from the level of each person (carrier or normal).
Table 1. Effect of Adjustment for Age and ABO Blood Group

<table>
<thead>
<tr>
<th>Discriminant (Adjustment)</th>
<th>Carriers</th>
<th>Normal Subjects</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Average Odds Favoring Carriership</td>
</tr>
<tr>
<td>Lab-specific (unadjusted)*</td>
<td>226</td>
<td>27:1</td>
</tr>
<tr>
<td>Lab-specific (age)*</td>
<td>226</td>
<td>22:1</td>
</tr>
<tr>
<td>Lab-specific (age, ABO)†</td>
<td>109</td>
<td>38:1</td>
</tr>
<tr>
<td>Universal (unadjusted)‡</td>
<td>196</td>
<td>11:1</td>
</tr>
<tr>
<td>Universal (age, ABO)‡</td>
<td>196</td>
<td>13:1</td>
</tr>
<tr>
<td>Lab-specific§ (unadjusted)*</td>
<td>126</td>
<td>26:1</td>
</tr>
<tr>
<td>Lab-specific§ (Age)†</td>
<td>126</td>
<td>26:1</td>
</tr>
<tr>
<td>Lab-specific§ (Age, ABO)‡</td>
<td>60</td>
<td>27:1</td>
</tr>
</tbody>
</table>

*Subsample consists of five laboratories that supplied a normal cohort.
†Subsample consists of three laboratories supplying ABO data and a normal cohort.
‡Subsample on which ABO data were available.
§Subsample consists of five laboratories providing a normal cohort.

in that laboratory’s sample. This had the effect of adjusting the carrier population of each laboratory to a mean of 0, while preserving the relative differences between carriers and normals. Age and ABO-adjusted universal discriminants were then computed in the usual manner with these data. The resulting average odds and misclassification rates are given in line 3 of Table 2. The performance of the “universal” discriminant was improved when adjusted for differences between laboratories in this manner (line 1 v line 3), although it does not reach the efficacy of the lab-specific discriminants (line 2).

The adjustment procedure was then repeated using the

Table 2. Effect of Adjustment for Laboratory Variation

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>N</td>
<td>Average Odds Favoring Carriership</td>
</tr>
<tr>
<td>Universal (Age, ABO)*</td>
<td>196</td>
<td>13:1</td>
</tr>
<tr>
<td>Lab-specific (age, ABO)†</td>
<td>109</td>
<td>38:1</td>
</tr>
<tr>
<td>Universal (age, ABO, carrier mean)*</td>
<td>196</td>
<td>19:1</td>
</tr>
<tr>
<td>Universal (age, ABO, normal mean)†</td>
<td>109</td>
<td>31:1</td>
</tr>
<tr>
<td>Universal (age)‡</td>
<td>331</td>
<td>11:1</td>
</tr>
<tr>
<td>Lab-specific (age)§</td>
<td>226</td>
<td>22:1</td>
</tr>
<tr>
<td>Universal (Age, carrier mean)‡</td>
<td>331</td>
<td>15:1</td>
</tr>
<tr>
<td>Universal (Age, normal mean)§</td>
<td>226</td>
<td>19:1</td>
</tr>
</tbody>
</table>

*Subsample on which ABO data were available.
†Subsample consists of three laboratories supplying ABO data and a normal cohort.
‡Total sample.
§Subsample consists of five laboratories providing a normal cohort.
mean of the normal subjects from each laboratory's sample to adjust that laboratory's data. The result is seen in line 4. The improvement in the performance of the "universal" discriminant is clear; the misclassification rate is slightly lower than that of the laboratory-specific discriminant (line 2). This is especially remarkable when one considers that the estimate of misclassification rate for the lab-specific variety is known to be biased downward. The average odds for the adjusted universal discriminant are comparable to those of the laboratory-specific discriminants for carriers (line 4 v line 2), although remaining somewhat worse for normal subjects. A clearer picture of the relative performance of these two discriminants may be obtained from the scatterplot of the odds they assign to carriers (Fig 1A). Points above the diagonal correspond to individuals to whom the universal discriminant assigns higher odds for carriership (and thus does a better job of classifying), whereas those below the diagonal are assigned higher odds by the lab-specific discriminant. Points in the upper right hand quadrant correspond to individuals classified correctly by both discriminants. Figure 1B is the corresponding plot for normals, but the interpretation must be reversed since the odds plotted are odds for carriership: points above the diagonal are now better classified by the lab-specific discriminant, and the points

![Fig 1](https://example.com/fig1.png)

**Fig 1.** Comparisons of the universal linear discriminant with the lab-specific linear discriminant (A, B), and of the universal ratio discriminant with the universal linear discriminant (C, D). All discriminants were adjusted for age and ABO blood type; the universal linear discriminant in A and B is normal-mean-adjusted, but that in C and D is not. Except as noted, each solid circle represents an individual. The coordinates are scaled in "odds favoring carriership," where 0.01 = 1:100 and 100.00 = 100:1. The diagonal indicates equal odds for both discriminants at any point, so that the location of a point with respect to the diagonal indicates which method was more efficient. The vertical and horizontal lines producing the four quadrants provide the opportunity to assess efficacy quickly, the upper right and lower left being most indicative. (A) Comparison of the universal and lab-specific linear discriminants for carriers. Note that the lab-specific discriminant classifies carriers overall slightly more efficiently than the universal. The points in the right upper quadrant are those classified correctly by both discriminants; the extreme upper right-hand point represents 84 carriers assigned odds >100:1 by both discriminants. The points in the left lower quadrant are those misclassified by both discriminants. (B) Comparison of universal and lab-specific discriminants for normal subjects. The high concentration of points in the lower right quadrant means that both discriminants are effective, lab-specific tests producing lower odds in most instances. (C) Comparison of the (universal) ratio discriminant with the (universal) linear discriminant for carriers. A moderate number of persons were incorrectly classified by both tests (left lower quadrant). The majority were correctly classified by both tests (right upper quadrant), the linear discriminant being generally more effective. More were correctly classified by the linear and incorrectly classified by the ratio (lower right quadrant) than were correctly classified by the ratio and incorrectly classified by the linear discriminant (upper left quadrant). The concentration of points along the margin of the right upper quadrant represent carriers whose odds were >100:1 with the linear discriminant and <100:1 with the ratio. (A similar but lesser tendency is seen in the right upper quadrant of A and the left lower quadrant of B). (D) Comparison of ratio discriminant and linear discriminant for normal subjects. The linear discriminant is clearly more effective overall. Points in the left lower quadrant are those correctly classified by both discriminants. Those in the left upper quadrant are persons correctly classified by the linear discriminant but incorrectly by the ratio. Those in the right lower quadrant were correctly classified by the ratio but incorrectly by the linear discriminant.
classified correctly by both discriminants are those in the lower left quadrant. The scatterplots reinforce the conclusions obtained from the average odds: the discriminants perform equally well on carriers, but on normal subjects the lab-specific discriminant has a slight edge.

That these results were not a peculiarity of the laboratories supplying the ABO data was verified by repeating the analyses on the entire sample, using age-adjusted- but not ABO-adjusted discriminants. The results (lines 5 through 8 of Table 2) were quite similar to those of lines 1 through 4. Here again, the best discrimination using the universal discriminant was achieved after adjusting the test values using the means of a normal cohort (line 8).

The effect of using alternative assays to measure F.VIII and VWF:Ag levels was then investigated. In Table 3, all VWF:Ag assays were done by Laurell’s electroimmunoassay method, whereas F.VIII was measured either by bioassay (F.VIII:C) or immunoassay (F.VIII:Ag). The pattern is not consistent. In Milan, for instance, better discrimination is obtained by using F.VIII:C than F.VIII:Ag, but in Malmo the reverse is true. In Leiden, the discriminant with F.VIII:Ag has better average odds, but the same misclassification rate, as that with F.VIII:C.

The utility of alternative assays for VWF:Ag is examined in Table 4, Milan providing the relevant data. Measurement of VWF:Ag by immunoradiometric assay (IRMA) or enzyme-linked immunosorbent assay (ELISA) did not basically improve the accuracy of the discriminant based on data obtained by EIA.

Finally, in Table 5, the relative accuracy of linear discriminants is compared with that of discriminants based on the F.VIII:C/VWF:Ag ratio. Linear discriminants are clearly superior to ratio discriminants in all comparisons, most strikingly in the proportion of false-positives (normal subjects misclassified as carriers). All the “universal” ratio discriminants performed quite poorly, the average odds in line 4 actually being <1:1. The latter anomaly is due in part to the presence of five carriers having a F.VIII:C/VWF:Ag ratio > 4 to whom the ratio discriminant consequently assigns extremely low odds for carriership. When these individuals are eliminated from the sample, the average odds for carriership improves to 2.9:1, still significantly worse than that for the linear discriminant. The superiority of the linear discriminant is also evident from scatterplots of the odds (Fig IC and ID).

DISCUSSION

We have found an effective “universal” discriminant that can be used in any laboratory. Although laboratory-specific discriminants are possibly more effective for individual laboratories, their greater effectiveness was found to be due almost entirely to the fact that laboratories differ significantly in their mean levels of F.VIII:C and VWF:Ag. The latter may result from genetic differences in local populations or systematic differences in laboratory technique. Whatever the cause, when these mean differences among laboratories are removed, the resulting “universal” discriminant is just as effective as are the laboratory-specific ones when judged by the criterion of misclassification rates and very nearly as effective when judged by the criterion of average odds.

We suggest, therefore, that the step-by-step procedure given in Appendix A, including the specific constants we have derived, can be used effectively for carrier detection in any laboratory if a local set of normal subjects is assayed for F.VIII:C and VWF:Ag against the laboratory standard, and their mean values are computed and used for adjustment as we have described. It is not necessary that each laboratory base its assays on the International Standard, unless there is the additional goal of comparing potencies between laboratories or combining data for subsequent calculations.

As detailed in our companion article,6 both age and ABO blood type have an effect on F.VIII:C and VWF:Ag levels; blood type has a greater effect than age. Although correction for these variables does not greatly affect the average efficacy of the discriminant, we recommend that it be done since it may have a substantial effect for some individuals and is, in any case, computationally trivial (Appendix A). Use of a truncated age range did not improve the efficacy of the discriminant.

There have been reports5,8 that determining F.VIII:C by immunoassay (F.VIII:Ag) gives better discrimination than does use of bioassays. Our data from three laboratories show that there is not a real difference between the utility of the

<table>
<thead>
<tr>
<th>Procedure and Laboratory</th>
<th>Carriers</th>
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<th>Normal Subjects</th>
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<tr>
<td></td>
<td>Average Odds Favoring Carriership</td>
<td>Misclassification Rate (%)</td>
<td>Average Odds Favoring Carriership</td>
<td>Misclassification Rate (%)</td>
<td>Total Misclassification</td>
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<tr>
<td>F.VIII:C Bioassay</td>
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<td></td>
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<tr>
<td>Milan</td>
<td>49</td>
<td>21:1</td>
<td>10.2</td>
<td>49</td>
<td>1:9.9</td>
<td>2.0</td>
<td>6/98</td>
</tr>
<tr>
<td>Leiden</td>
<td>41</td>
<td>46:1</td>
<td>7.3</td>
<td>16</td>
<td>1:18</td>
<td>0</td>
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</tr>
<tr>
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<td>12:1</td>
<td>19.5</td>
<td>30</td>
<td>1:2.8</td>
<td>6.7</td>
<td>10/71</td>
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<tr>
<td>F.VIII:Ag immunoassay</td>
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<td>41</td>
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<td>7.3</td>
<td>16</td>
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<td>6/71</td>
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</tbody>
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All VWF:Ag assays were done by Laurell’s electroimmunoassay method. Specific age-adjusted discriminants were calculated for each assay from each laboratory.
two methods. Some laboratories are better with one assay, some with another. Each laboratory should make a choice based on its own experience.

One laboratory provided data that allowed us to examine not only two methods for assaying F.VIII:C but also three methods for assessing VWF:Ag. It was clear that bioassay of F.VIII:C was more efficient in this laboratory than was immunoassay of F.VIII:Ag, but the methods of assaying VWF:Ag did not differ appreciably; Laurell’s electroimmunoassay for VWF:Ag was as satisfactory as the IRMA or the ELISA.

The ratio method for assessing potential hemophilia carriers has been widely used because of its computational simplicity. We compared the utility of linear discriminants with the utility of ratios, both as universal and as lab-specific discriminants, adjusted for age only and for age plus ABO blood type. In every instance, the linear discriminant outperformed the ratio. The ratio method not only had significantly worse average behavior but also tended to make serious misclassification errors, assigning extremely high odds for carriership to some normal subjects and vice versa.

What we have described is the analysis of a complex clinical laboratory experiment designed to determine the best methods for determining the genotypes of hemophilia A carriers from their phenotypes. Now that there are methods for determining the genotypes of carriers directly from their DNA, however, one may ask whether the phenotyping methods are obsolete. As discussed in a recent review of the advantages and limitations of phenotypic and genotypic methods, there is a place for both, since there are occasions when the DNA method cannot be applied. Furthermore, even when the DNA method is applicable, the information obtained concerning genotype is often probabilistic (since the most informative polymorphic markers show a non-zero probability of crossover with the disease) and, when it is combined with information derived from the older method,
discrimination is improved over that obtainable by either method separately.4

In summary, our observations have led us to make the following recommendations. First, when possible, a laboratory should endeavor to collect enough data on its local carrier population to permit the computation of its own local discriminant. When this is not possible, however, the “universal” discriminant developed here can be used as described in Appendix A, provided that a set of normal females has been assayed for F.VIII:C and VWF:Ag against the local standard and their means have been used in the adjustment procedure as described. A new group should be assayed against each new working standard.

Second, in all cases, ABO data should be collected for inclusion in the discriminant. Age data, though probably somewhat less important, should be noted as well.

Third, choice between the more traditional assays and the newer assays depends entirely on the specific experience and expertise of the laboratory. It is clear that the traditional F.VIII:C and VWF:Ag assays are fully useful in practiced hands.

Last, the ratio method of discrimination should be avoided. Its chief merit, computational simplicity, can no longer be considered important since personal computers and statistical packages are now widely available. The method described in Appendix A can be easily used on a programmable calculator.

APPENDIX

Procedure for computing probability of carriership of hemophilia A. Measurement of F.VIII:C and VWF:Ag may be done by local “in-house” methods. In using the following procedure, it is not necessary to convert from local “working” units to international units, because the adjustment procedure in step 2 will automatically correct for differences in the scale of measurement. (If a laboratory wishes its reported assay values to be comparable with those of other laboratories, it should of course calibrate its working standard against the international standard.) Assays on the normal and test subjects should be conducted under conditions as nearly identical as possible, using the same working standard. The sample on which the universal discriminant was based did not include women who were pregnant or using contraceptives; consequently, the following procedure may not be applicable to such individuals.

9. Peake IR, Newcombe RG, Davies BL, Furlong RA, Ludlam CA, Bloom AL: Carrier detection in haemophilia A by immunologi-
cal measurement of factor VIII related antigen (VIIIRAg) and factor VIII clotting antigen (VIIIICAg). Br J Haematol 48:651, 1981
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