Hints to blood groupers, 1950

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Sixty years ago, the premier blood grouping laboratory was that of Robert Race in London. Agglutination tests and blood grouping had provided breakthroughs in immunology, genetics, and the solution of clinical problems. The significance of immunohematology was recognized by the clinical hematology community as a potent force in the expanding field of disorders of the blood and blood-forming organs. The instructions by Race to his London workers entitled *Hints to Blood Groupers* provide a picture of the immunohematology laboratory even before automation and differed slightly from the American techniques that derived from Landsteiner. Before agglutination is replaced in the near future by the emergence of molecular methods, the detailed method of a superb laboratory is recorded.

The agglutination test, which has been the basic tool of blood grouping for a century, has begun its inevitable advance into obsolescence. When the molecular methods that can identify single-nucleotide polymorphisms (SNPs) are adapted to routine automated blood typing, poor old agglutination will be gone. Many of the things that disappear from the information available in our civilization carry valuable knowledge and wisdom into oblivion. We present for the record a story of the agglutination method by which hundreds of millions of times, perhaps a billion times, donor and patient were matched, and their polymorphisms were transfused from one human being into another over the past 100 years.

**HOW IT BEGAN**

In the first half of the last century, all of the discoveries of the then new immunology called blood grouping depended on the agglutination test. Landsteiner's accidental discovery in Vienna of agglutination between the bloods of normal people in 1900 led to the discovery of the ABO system. He moved to New York and with his pupils Levine and Wiener described MN and P. All together their discovery of Rh in 1939 and 1940 uncovered new relationships in immunology, genetics, and human disease and was ranked as one of the most important medical events in the first half of the 20th century. Blood group studies blossomed in Boston, Buffalo, Dallas, Chicago, and Winnipeg that captured the interest of the clinical hematology community. One of us (TJG) had been a research fellow with America's then premier hematologist, William Dameshek, from 1941 to 1942 and participated in studies that identified the significance of the hemolytic syndrome and bilirubin in the clinical picture of erythroblastosis.

**ENTER ROBERT RACE**

The only European speaker at the 1946 meeting was Robert Race of the Lister Institute in London. The center of power in blood grouping was ready to shift from New York to London, and Race's laboratory became the place to which the interesting and new problems went to begin the second half of the century of immunohematology. Race, with his associate Ruth Sanger, published the work of that laboratory and of the world in 1950 in their book, *Blood*.
All of it was based on agglutination technology. Unlike later editions, the first printing of the book did not include a chapter on method, although much technical information was scattered through the text. An earlier set of directions for Race’s own laboratory staff exists which he titled *Hints to Blood Groupers*. Despite the informality in Race’s writing to his coworkers, that document illustrates precise concepts and is here published solely for the enjoyment of older technologists, the edification of those who have known only automated methods, and as a record for the future of what agglutination was all about.

The entire Lister Institute document is transcribed here, corrected by some handwritten notes that had been inserted into this copy, perhaps by Race or by Sanger, before it was given to one of us (TJG) by Race on a 1954 visit to the Lister. The addendum has been placed here first because it states why the London work is so very important to the knowledge and history of blood grouping. Race’s remembered grace can be seen in his “hints” and no attempt has been made to change spacing, spelling, or punctuation into American English in the following verbatim transcription:

**HINTS TO BLOOD GROUPERS**

Ruth and I have found the foregoing details not only helpful but necessary in blood grouping. Blood grouping in which errors do occur is a waste of serum, for the correct results in which errors are set will not be believed. Errors that get beyond these walls are more than a waste of serum they are very dangerous. Our groupings are accepted without question because of our prestige, remember the responsibility of this for an error could easily be lethal.

**PREPARATION**

Clear adequate room on the table so that there is space to move things about delicately without bumping unnecessary obstacles.

Get out the necessary sera and put away the rest.

Use shallow blocks for cells and sera so that you can see what the label says; that is up to the time when you place the cell and serum tubes in the test blocks.

Use a separate shallow block for the cells in the immediate test, do not have odd cells in the block at the same time. Use a separate shallow block for the serum about to be used in this immediate test, do not have odd sera in the block waiting to be used in say the MN tests next on the list. If as many as 12 sera are to be used take 2 shallow blocks, so that 11 and 12 are in 11 and 12 and not confusing 1 and 2.

Use beakers and round glass dishes for saline, but flat dishes for water. Never put water in a beaker or in a round glass dish.

The pencil must be reasonably sharp and the rubber found. (If later the rubber has to be looked for or the pencil sharpened a dangerous diversion will be caused.)

Label the tubes of cells slowly, so that they can be read. For members of the Unit or A.E.M. or W.T.J.M. or E.W.I. initials are appropriate. Initials will not do for anyone else, nor will Christian names (with the exception of Stella which is an uncommon name). Neither “Drury” nor “Wedge” alone will do for there are two of each in the Institute. Put the day of the week or date on the tube. If the cell suspension is in albumin put “alb”, if serum-albumin put S/A.

Put a small rubber band round cell and serum tubes – it helps to keep the label on but it has a more important function. By means of the band the serum or cell tube can be raised in the test block and with the label half facing away so that the mark on the pipette is not lost in deep darkness but is visible against the white background of the back of the label.

**SETTING UP THE TESTS**

Head the sheet with the date, then with the work, e.g. “Lister People” or, if a special sample is being tested, give the individual name and the name of the doctor who sent it and the place where he lives. Note the date of sending if available, note date of arrival, say whether the samples looked O.K. or not. Another example – “Finger pricks Fountain 9.10.50. Collected by June 10.10.50. All good samples.”

Draw up the protocol; this must be a complete and exact (except for the mirror imagery) plan of what you are going to do. Always write 1 · 2 · 3 · 4 etc. and a.b.c. etc at the ends of appropriate columns and rows. This must be done in every case but it is
clearly indispensable when the pattern is symmetrical. Write down the block numbers before distributing the precipitin tubes. Write down slowly all the details on the serum tube, e.g. “Bastow abs. 12, 10.47.” and above it write “anti-C”, or, another example “Eaglestone anti-Lu”. In two years time I certainly will not remember that Eaglestone was an anti-Lu”. If there is not much room perhaps “C” or “Lu” would do, leaving out the anti but keeping the dash. Write down slowly (so that they can be read) the names of the cell donors. If they are in a, b, c rows put a, b, c etc to the left of the protocol lines. If it helps you can put 1-10 to the left of the names. This is not the 1-10 of the columns however and must be distinguished. If the protocol extends over 2, or more, blocks then draw heavier lines to indicate change of blocks. Put block numbers in the proper place in the protocol. It is not good enough to put “blocks 1, 2 and 3” under the protocol. If it is a titration put the block numbers far enough to the right to avoid their confusing the scores which may subsequently be entered. If, during testing, one tube of “Bastow 1/1 Nov. 47” runs out and a fresh tube is started put a line in the protocol to mark where the change has occurred. Blocks and tubes. Have your initials on the front of blocks only. Have your initials facing you. Put your blocks away in order and with the initials to the left or right or how you will, but all the same. Fill the blocks with tubes according to the protocol. Use upside down tubes wherever practical to mark blanks. If you are starting a titration at 1/2 it must have been recorded on the protocol. Make tube 1 1/2 if you wish the titration to go to 1 in 1024, that is if you need all the holes. If you do not need all the holes it is much safer to have 1/2 in its usual place, in hole 2; in which case fill hole 1 with an inverted tube. There are various reasons why it is bad to have empty holes in the line. Suppose you want to test No. 1, 4 and 7 with say alpha-one and you want to add a column to a previous protocol (of say cells 1-10 v anti-A and anti-B). Then place tubes 1, 4 and 7 in a block and fill in, with upside down tubes, holes 2, 3, 5, 6, 8, 9 and 10. Pipettes make sure mark is clear especially before using albumin. Sera. Remove the tubes from the shallow block into the test block, one by one using only one hand, checking that it is as already written down on the protocol. If there is no vacant row or column in the test block, have an empty deep block for the serum lined up behind the test block, or to the right of it as the case may be. After each serum is pipetted move the tube at least one row or column – into another deep block in alignment if no space is left to move into in the test block. Cells: Remove the cells from the single row of their shallow block to the columns or rows of the test block. Do this one at a time using only one hand, checking each one by the protocol. After each cell suspension is pipetted move it at least one row or column, into another deep block in alignment, if no space is left to move into in the test block. When trying to make comparable cell suspensions pick out tubes of the same bore. The four inch absorption tubes if carefully selected are good for this purpose. The cell suspensions should be inspected all together in an open rack. The suspensions when satisfactory should be decanted into postal tubes. Controls. Record the appropriate groups of the control cells, e.g. MN or Rh etc. It makes the protocols intelligible later, without having to refer to a list which may by then be superannuated and difficult to find. Never trust your memory of a person’s group (it would of course usually be right, but it has been proved fallible). It is perhaps going too far to look up say Ruth’s ABO group but it is clearly necessary to look up her S group. Always control every serum. If say one of the controls is, unavoidably, elderly, note this down (subsequent inspectors of your protocols should know this). Shuffle up control cells of different groups in serum investigations, to make it easier
to forget the order of the cells; e.g. do not put all the rr cells together.

**Incubator.** Place blocks end on in the incubator with your name to the left.

**Coombs test.** Get everything poised before starting. Write up protocol first as usual. Arrange tubes so that they are in rows not columns. Write the number with a wax pencil near the top or middle and near the bottom of the tube. (one or the other may come off). Rows should not be used, for a . b . c etc are not so clear as 1 . 2 . 3 when written in wax on the small rounded surface.

Make a tick on the sheet, near the time recording, as you return from switching on the centrifuge for each washing. You will not otherwise wash three times without fail.

Have some systems for putting tubes into centrifuge buckets. Scrub the tile twice with nail brush and soap and dry with a very clean towel. Do not touch the surface after washing. Never set out more than 10 tests on the tile at a time. Take the times of reading with a stop watch which is started at the time of mixing the cells and the anti-globulin.

**Reading.** It is essential that there should be no talking and that the door should be shut. Have a scorer if a silent and reliable one is free. Never turn round to welcome anyone until you have studied, and recorded, in your own time, the slide you have made. Never answer the phone until you have, in your own time, studied and recorded the slide you have made. Do not answer anyone who speaks at an inconvenient moment. Do not answer the phone if it is inconvenient.

Have a standard arrangement of all things necessary for the reading and make room for all things. Do not for example have the board uneasily couched on an orange.

Give your mind to recording your results, try to concentrate solely on getting the pluses, weaks etc. into the right place. This will take all your powers of concentration. Do not let your mind race ahead to the interpretation or sideways to anything else.

When reading without a scorer cover with a postcard the names, but of course leaving 1 . 2 . 3 or a. b. c etc. shewing. This eases the difficulty of trying to be fair and unbiased.

**Interpretation.** Make your diagnosis and write it down on the left, by the person’s name. Later cover up this diagnosis and make it again, or better, get someone else to make it.

**Checking.** Look carefully at your results and repeat any dubious ones, as soon as possible. Repeat again if necessary and make new protocols for the repeats. It is no good saying you have repeated it and get the same results. That would soon be forgotten if not written down. If say an anti-c is giving ? or ?w results with one sample it is not good enough to put the serum up subsequently with a known RrR and if that is ?w to say that the sample is also RrR. The doubtful sample should be put up with RrR and RrR at the same time, and, if there is still doubt the sample should be tested with further anti-c sera.

**General serological behaviour.**

Never offer to score for a person. Ask a person to score for you if you think fit.

If you want to speak to a person working try to see when it will cause least disturbance, if you cannot see ask her to tell you when she can speak and pretend to be interested in something else in the room; do not stand within the worker’s vision.

Write legibly on all tubes - both cell suspensions and sera for the deep freeze. It is not hard and does not take long. If, for example, Davis is not written very clearly it is indistinguishable from Dawes.

Serum for the deep freeze should be labeled very clearly, with date. If it is a name like Smith initials should be added. Otherwise they are no use in months to come.

Dates are important in all respects, e.g. and I.C.T. protocol should have a record of the date on which the A.H.G was diluted.

Never make copies of protocols unless a duplicate has to be sent somewhere, and if this is known beforehand a carbon is better. They must be clear enough the first time, and copying only leads to errors.

Return letters and protocols without fail, before they are forgotten.
Do no hesitate to come and ask for advice. Put only current boxes in the −20° frig; all must have strong elastic bands round them. Boxes must not interfere with access to the other half of the frig. Keep your own half neatly stacked to avoid annoying others.

Keep the domestic refrigerator tidy, do not put in a huge wooden block for 2 tubes that could go in a tin. If food must be put in, put it discreetly out of the way. It tends to annoy.

Always fill tubes with water at the end of the day, the sooner the better.

TRANSCRIBERS’ COMMENTARY

The article makes reference to A.E.M. That identifies Mourant, W.T.J.M. is Morgan, and E.W.I. is Ikin. The rubber would be the eraser for the sharp pencil.

Some of the instructions to staff would not be tolerated today such as not to have anything “couched on an orange” or “keep the domestic refrigerator tidy” and “if food must be put in, put it discreetly out of the way.” More useful today in the text are counsels to seek an assistant who is “silent and reliable,” to “not answer the phone if it is inconvenient,” and, best of all, not to “hesitate to come and ask for advice.”

Other older methods

Some differences between English and early American methods are apparent. At that time, American laboratories were testing by methods of the manufacturers of reagent sera such as John Elliott, who gave more emphasis to slide techniques. His collaborator, Jack Griffitts, said that those methods, described for their Dade Reagents, went into the procedures manual of the Florida Association of Blood Banks, which in turn was used to formulate the AABB technical manual in 1953.7

The AABB Manual called for ABO grouping to be done on a glass slide using whole blood and the tube method was for secondary confirmation. The “tile” to which Race refers was a 3- or 4-in. square slab of glass or white stone that has now been superseded by the “microplate.” A “block” was a drilled wooden tube holder, akin to today’s test tube rack.

Since agglutination requires contact between antibody-coated cells, Race used prolonged incubation of test sera and cells in test tubes to create that event and did not describe centrifugation of the tubes. Americans, always being in a hurry, centrifuged tubes when they were used and then needed to resuspend the cell sediment. That act of resuspension was an art and not a science and led to discrepant findings depending on how vigorously it was done. Results depended on whether the artist was a shaker or a tapper, a rotator, an up-twirler, a bench-banger, or even more innovative. Nevertheless, the descriptive grading and scoring of agglutination as used by Race and Sanger were accepted generally in American systems.

A Russian method mixed cells and reagent on a white dinner plate decorated with blood grouping symbols. A Canadian method allowed cells to drift down through reagent in capillary tubing to develop precipitation patterns depending on the presence and size of any agglutinates that might be formed in transit. A card impregnated with antibody was introduced in Denmark and tried by the American Army. A variant of the latter method in which antisera and cells were mixed on the blood donor’s record card allowed the card to be stored as a permanent record in a filing cabinet, much to the joy of cockroaches.

Agglutination and molecular testing

In the fourth edition of Blood Groups in Man, Race and Sanger withdrew from the methods field, saying that they were leaving out that information in order to save space and because tests “can be varied in so many ways, and yet be reliable.” Instead, they gave the reader more than seven pages of references to published method.8 The technical manual of the AABB was not referenced.

One-hundred years after Landsteiner used agglutination to test the blood of his healthy colleagues for the antibodies seen in disease and as a result came upon the blood groups by accident, agglutination persists as the premier method of ensuring safe transfusion. Today’s automation can use robot hands to ease what Race called “the difficulty of trying to be fair and unbiased” but the adequacy of agglutination testing in typing donor blood has been challenged in discussion of the antigenicity of the DEL factor of the Rh system. DEL, well recognized by molecular testing, could be described as a weak, weak D. Its possible clinical significance has raised interest in Europe in extension of molecular diagnostics to the typing of donor blood.9 One-hundred years of testing by “varied, yet reliable” agglutination will become only a memory, but a good one.

REFERENCES
