Although Rh already seemed complex by the 1940s, far more was to come. Myriad additional Rh antigens and serologic subtleties were revealed. Many fit smoothly into neither the Wiener nor Fisher-Race schemes, and convoluted machinations would sometimes be offered to explain them. Often the complexities escaped the understanding of all but a very few “Rh experts” (and we sometimes had our doubts about them). Ultimately, both Wiener and Fisher-Race approaches would prove incorrect, replaced by a two-gene theory of inheritance and a better understanding of the biochemical nature of the Rh antigens. (See Chapter 27.) These would not come for many years, but some of the peculiar intricacies of Rh had already started to appear.

D

Differences in expression of the D antigen were noted as early as 1944, when Wiener reported what he called “intermediates,” reactive only weakly with routine anti-D; he showed that such weak expression was inherited. In 1946, Fred Stratton, from the Blood Transfusion Centre in Manchester, England, reported red cells with weaker-than-expected expression of the D antigen. These red cells were agglutinated by some anti-D, but not by others. Family studies suggested the weak D antigen was an inherited characteristic. That same year, Sheila Callender and Rob Race had discovered the antigen $C^{w}$, which seemed to be the product of a third allele of $C$ and $c$. (See Lutheran, Chapter 27.) Based on their conclusions, Stratton similarly proposed a third gene allelic to $D$ and $d$, producing a weaker antigen. On advice from
Building on methods used to isolate T and B lymphocytes (using surface-bound antibodies), they successfully affixed blood group antibodies onto the wells of plastic microplates. When suspensions of red cells were added, those positive for the particular antigen would attach to antibody over the entire surface, forming a film, or effacement, of red cells. When the microplates were centrifuged, these bound cells remained firmly attached, while unbound, antigen-negative red cells pelleted to the bottom of the microwell. Alternatively, the wells could be coated with red cells (using chemicals, such as poly-L-lysine or gluteraldehyde, or certain sera containing antibodies of broad specificity) so that when cells of known antigen phenotype were used, plates could act as panels for antibody detection and identification. With certain modifications, antiglobulin tests could be performed.

The DOT DAT

Combining solid phase with immunoblotting techniques, Plapp attached antibodies onto small, nylon membrane squares, which were attached to plastic handles. After quickly wetting the sticks in saline, drops of blood were applied to the squares. After a minute, the sticks were swirled in saline. In a negative test, the red cells were washed away, but in a positive test, they adhered. Such dipsticks offered a potentially easy typing system, especially suitable for non-laboratory settings. A variation allowed direct antiglobulin testing—which Plapp dubbed the DOT DAT, a play on the DOT BLOT, a simplified method in molecular biology to detect various biomolecules.

Left, solid-phase adherence assay for red cell antigens A, B, and D; right, solid-phase antibody detection test for red-cell-related antibodies (Courtesy of AABB)
Test Tubes

Tube tests were recommended by Landsteiner, predominated in blood group serology for most of the 20th century, and are still widely used. Tubes were particularly helpful when testing multiple samples, limiting the drying that occurred on slides and allowing longer incubation. Hemolysis was more easily observed. Tube tests could readily be converted from one temperature or phase to another. They were adaptable to centrifugation and to the saline washings needed for the antiglobulin test. By 1955, tube methods were so standard that Dunsford and Bowley's text, *Techniques in Blood Grouping*, already referred to them as the "classical tube technique." Yet the ways in which tube tests were performed evolved over the decades.

Initially, the test tube served mainly as a reaction chamber. After incubation, a glass pipet was inserted into the test tube and a small volume of red cells was extracted, spread onto a slide, and examined microscopically. Sometimes results were observed while cells were still in the tube. After the red cells had settled to the bottom of the tube, a macroscopic reading was made. The edge of the settled button of red cells was telling: a smooth edge indicated a negative result, while a rough or serrated edged pointed to agglutination. Tube racks were sometimes suspended over magnifying mirrors so that the reflected tube bottoms could be easily viewed.

Small tubes were initially preferred. Schiff's 1942 book recommended "micro" tubes about 35 mm long (about 1.4") with an inside diameter of 2.5 mm (about 0.1"). Tests in these tubes used only 0.04 mL of antiserum and an equal volume of red cells, which were added atop the serum using a very fine capillary pipet. The tubes were inserted vertically into plasticine (modeling clay), then sat until the red cells settled to the bottom. As an alternative, the tubes could rest in specially designed racks; these offered the advantage of keeping the outside of the tubes clean and allowing full view of the settled red cells.

"Precipitin" tubes were another popular choice, especially in Britain. Routinely used in bacteriology labs (from which they were often purloined for the blood bank), these were about 7 × 50 mm (0.25" × 2.0") rounded on the bottom and with no lip at the top. They were sometimes covered with small glass caps during incubation at 37 C to prevent drying and contamination. They fit closely together in the tube racks, so that a skilled hand could

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*The exact size varied. Some were 0.50" × 3" or 0.375" × 3".*
In 1937, Bernard Fantus established what is often cited as the first blood bank, at the Cook County Hospital in Chicago.\textsuperscript{20-22} The facility featured bottled blood and refrigerated storage. Fantus coined the term “blood bank”—based on the manner in which it exchanged credits, had “withdrawals,” and encouraged “deposits”—and popularized the concept. Others, in Russia, Canada, Spain, and at the Mayo Clinic, likely preceded Fantus’ operation in various aspects.\textsuperscript{23-26}

But if Cook County Hospital is accepted as opening the first blood bank, then Sigfred (“Red”) Moinichen must surely be considered the first blood bank technician. He was at work in the hospital’s laboratory when the blood bank began and supervised its operations for the remainder of his career. He became active in the AABB and Illinois Association of Blood Banks and was a founder and first president of the Greater Chicagoland Corpuscle Council (the local “antibody club”). A devoted educator, he was also a keen photographer and used his many pictures in his teaching. Unfortunately, most of his photos were destroyed when his home’s basement was flooded.\textsuperscript{27} (E-mails from Peter Moinichen to SRP, January 2013.)
**Recommended Study Aids for Blood Bank Exam (1954)**

The blood bank examination was drawn primarily from the AABB's *Technical Manual and Procedures*, but several additional references were recommended:

- *The Human Blood Groups*, by P.H. Andresen (1952)
- *Standard Values in Blood*, by Errett Albritton (1952)
- *The Foetal Circulation*, by Alfred Barclay, Kenneth Franklin, and Marjorie Prichard (1945)
- *Blood Clotting and Allied Problems*, by Joseph Flynn (1952)
- *Medico-Legal Blood Group Determinations*, by David Harley (1948)
- *The Rh Factor in the Clinic and Laboratory*, by Joseph Hill and William Dameshek (1948)
- *Proceedings of the Third International Congress of the International Society of Blood Transfusion* (1951)
- *Haemolytic Disease of the Newborn*, by Margaret Pickles (1949)
- *Blood Grouping Technic*, by Fritz Schiff and William C. Boyd (1942)
- *Practical Blood Grouping Methods*, by Robert Wall (1952)
- *An Rh-Hr Syllabus: The Types and Their Applications (Modern Medical Monographs)*, by Alexander Wiener (1954)

**The “First SBB”**

One of those nonregistered blood bank workers was Shirley Busch. She had received a degree in bacteriology from the University of Illinois in 1939. With World War II under way, Busch joined the US Navy WAVES (Women Accepted for Volunteer Emergency Service), working as a laboratory officer, primarily at the Naval Hospital in Long Beach (where, she recalled, they made their own in-house typing reagents and had all the latest equipment—including a centrifuge to spin specimens and separate serum from red cells). After the war, she earned a master's of public health degree from Columbia University and was then hired as supervisor of the Blood Center of Mount Sinai Hospital in Chicago in 1951, working for Israel Davidson and Kurt Stern. Because her degrees were not specifically in blood banking, Busch wanted some way to document her abilities. Being in Chicago was a big advantage to Busch. Blood banking was progressive in the city, and educational opportunities for blood bank workers surpassed those in many other cities. Both ASCP and AABB had offices in Chicago; Stern and Davidson were leaders of both organizations and Busch was quickly involved, too. When she learned that there was under consideration a new designation of “Specialist in Blood Banking,” beyond the regular blood bank certification and “intended to recognize a superior category,” she was quite interested. “Specialist” certifications had already been granted by ASCP, beginning in 1953, in bacteriology and in chemistry; they were intended for those who held either a master's or PhD degree. Busch was the first to apply for one in blood banking.

Requirements for the specialist designation were not entirely clear and at times Busch was as much a part of deciding how things would proceed as were her examiners. Written and practical exams were required; Busch passed these in 1957. She was then informed by Griffitts (of the AABB Education Committee) that there would be an oral examination as well, to be given at the next AABB Annual Meeting. Griffitts did not say who her examiner would be, only that it was a “very fine person” and that it would be “a pleasure for you and him to get together.”

There was confusion as to what to call the new designation. Busch first received a certificate designating her as “Technical Specialist in Blood Banking.” The AABB Bulletin referred to her as “Specialist B.B.1.” About a year later, a second certificate arrived, titling her “Specialist in Blood Bank Technology.” It was a while, though, before anyone else attempted the new certification. Busch recalls being contacted to ask her permission to use the SBB designation for other people, as if she held the rights to it. (Interview with Shirley Busch by SRP, April, 2006.)