CHAPTER

fifteen

Alternative Technologies and Automation in Routine Blood Bank Testing

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OBJECTIVES

On completion of this chapter, the learner should be able to:

1. Define the principles of gel and solid-phase technology.
2. Describe the test reactions and methods of grading reactions for each technology.
3. List the advantages and disadvantages of each technology.
4. Compare the two technologies in terms of equipment, test reactions, procedures, sensitivity, and quality control.
5. Discuss the automated equipment that is available for each technology.

Introduction

Previous chapters have discussed ABO and Rh typing, direct antiglobulin testing (DAT), and antibody detection and identification procedures based on routine tube testing techniques. In response to the pressures of current good manufacturing practices, two alternative technologies, the gel test and solid-phase assays, have emerged to provide accurate, reproducible blood bank testing. These technologies offer increased safety provided by plasticware and decreased biohazardous waste. In addition, automation decreases the opportunities for human errors and frees laboratory personnel to perform other tasks. This chapter presents the history, basic principle, test reactions, and advantages and disadvantages of each technology and discusses the automation of the testing technology.

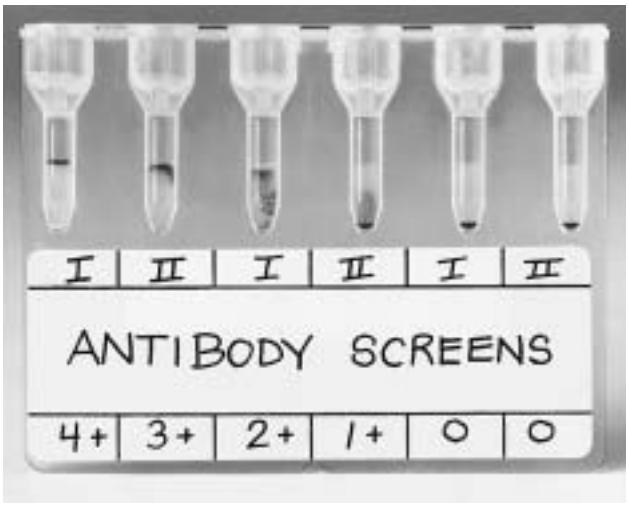
Gel Technology

History

In 1985 the gel test was developed by Dr. Yves Lapierre of Lyon, France.[1] Various media, including gelatin, acrylamide gel, and glass beads, were investigated in an attempt to trap agglutinates during a standardized sedimentation or centrifugation step. Gel particles appeared to be the ideal material for trapping agglutinates, and this discovery led to a patented process for the separation of red blood cell (RBC) agglutination reactions. In addition, it was discovered that antiglobulin testing could be performed without multiple saline washes to remove unbound immunoglobulin and that antiglobulin control cells were not needed to confirm the presence of antiglob-

ulin reagent in negative tests. Compared with traditional tube technology, the gel test provides a more stable endpoint and a more reproducible result. This improvement reduces the variability associated with the physical resuspension of RBC buttons after centrifugation and the subsequent interpretation of hemagglutination reactions.

In 1988 Dr. Lapierre aligned with DiaMed A.G., Murten, Switzerland, for the commercial development and production of the gel test in Europe. In September 1994, Micro Typing Systems (MTS) Inc., Pompano Beach, Florida, an affiliated partner of DiaMed A.G., received a Food and Drug Administration (FDA) license to manufacture and distribute an antiglobulin anti-IgG gel card and a buffered gel card in the United States. In January 1995, Ortho Diagnostic Systems Inc. (ODSI) and MTS signed an agreement giving ODSI exclusive rights to distribute the gel test in North America. In March 2002, Ortho-Clinical Diagnostics, Inc. acquired MTS, which is now known as Micro Typing Systems, a wholly owned subsidiary of Ortho-Clinical Diagnostics, Inc. This gel-based test is named the ID-Micro Typing System™.[2]



■ **FIGURE 15–2** The gel card (microtubes are used instead of test tubes).

Principle

The gel test, which is performed in a specially designed microtube, is based on the controlled centrifugation of RBCs through a dextran-acrylamide gel that contains predispensed reagents **(Fig. 15–1)**. Each microtube is composed of an upper reaction chamber that is wider than the tube itself and a long, narrow portion referred to as the column (see **Fig. 15–1**). In the gel test, a plastic card with microtubes is used instead of test tubes **(Fig. 15–2)**. A gel card is approximately 5 × 7 centimeters and consists of six microtubes. Each microtube contains predispensed gel, diluent, and reagents if applicable. Measured volumes of serum or plasma and/or RBCs are dispensed into the reaction chamber of the microtube **(Fig. 15–3)**. If appropriate, the card is incubated **(Fig. 15–4)** and then centrifuged **(Fig. 15–5)**.

The reaction chamber is actually a miniature test tube, providing an area for the sensitization of RBCs (antigen-antibody binding) during incubation. The column of each microtube contains dextran-acrylamide gel particles suspended in a diluent or reagent. The shape and length of the column provides a large surface area for prolonged contact of the RBCs with the gel particles during centrifugation.

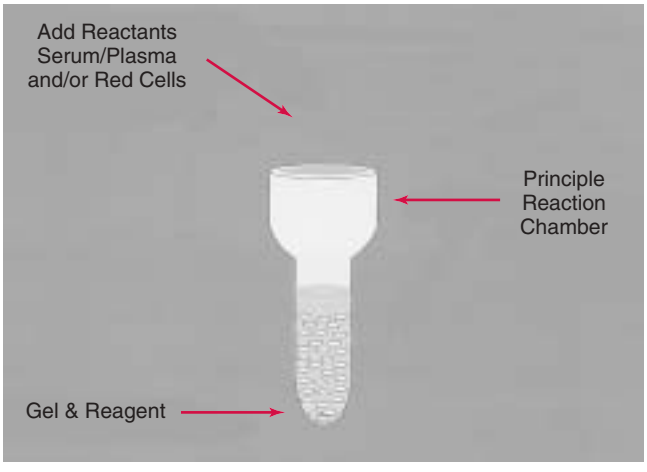
The gel particles are beads of dextran-acrylamide that make up 75 percent of the gel-liquid mixture that is preloaded into each microtube.[2] The gel particles are porous, and they serve as a reaction medium and filter, sieving the RBC agglutinates according to size during centrifugation. Large agglutinates are trapped at the top of the gel and are not allowed to travel through the gel during the centrifugation of the card **(Fig. 15–6)**. Agglutinated RBCs remain fixed or suspended in the gel, while unagglutinated RBCs travel unimpeded through the length of the microtube, forming a pellet at the bottom following centrifugation.

Unlike agglutination in the traditional test tube hemagglutination method, the gel test reactions are stable, allowing observation or review for up to 3 days.

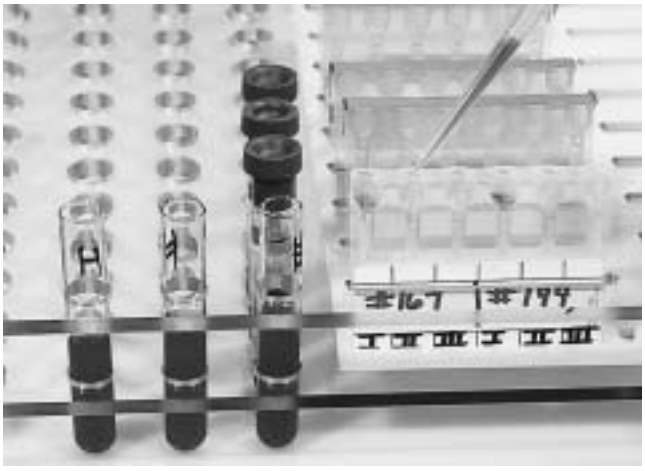
Test Reactions

Agglutination reactions in the gel test are graded from 1+ to 4+ (including mixed field), just as the reactions in test tube hemagglutination technique are graded **(Fig. 15–7)**.[2]

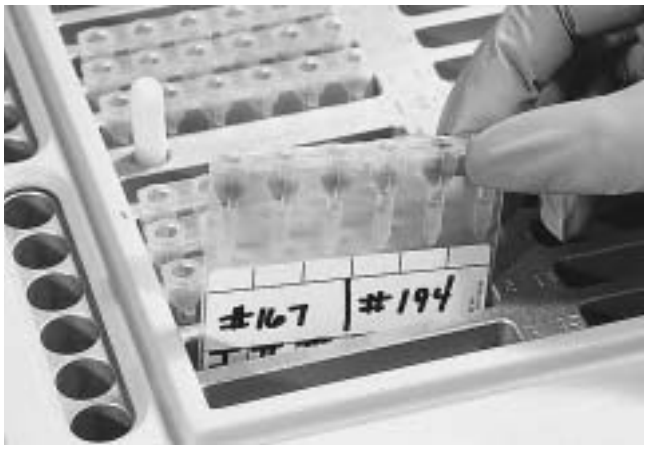
In the gel test, a 4+ reaction is characterized by a



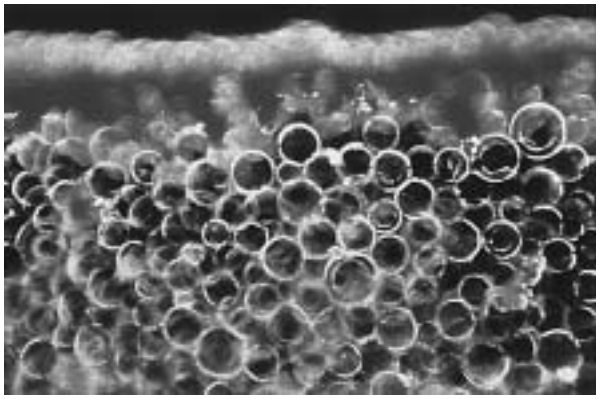
■ **FIGURE 15–1** Illustration of gel microtube.



■ **FIGURE 15–3** Pipetting into gel card microtubes.



■ FIGURE 15–4 Incubation of gel cards.



■ FIGURE 15–6 Magnified photograph of agglutinated RBCs trapped above the gel matrix.

solid band of agglutinated RBCs at the top of the gel column. Usually, no RBCs are visible at the bottom of the microtube.

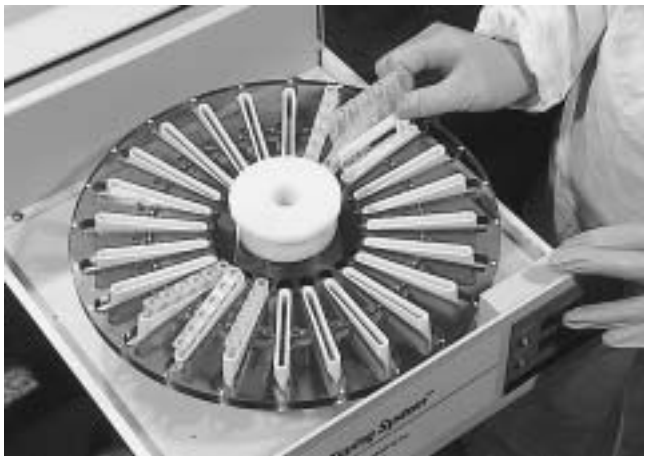
A 3+ reaction is characterized by a predominant amount of agglutinated RBCs near the top of the gel column, with a few agglutinates staggered below the thicker band. The majority of agglutinates are observed in the top half of the gel column.

A 2+ reaction is characterized by RBC agglutinates that are dispersed throughout the gel column, with a few agglutinates at the bottom of the microtube. Agglutinates are distributed throughout the upper and lower halves of the gel.

A 1+ reaction is characterized by RBC agglutinates that are predominantly in the lower half of the gel column, with some RBCs at the bottom of the microtube. These reactions may be weak, with only a few agglutinates remaining in the gel area just above the RBC pellet at the bottom of the microtube.

In a negative reaction, the RBCs form a well-delineated pellet at the bottom of the microtube. The gel above the RBC pellet is clear and free of agglutinates.

Mixed-field reactions are characterized by a layer of agglutinated RBCs at the top of the gel accompanied by a pellet of unagglutinated cells at the bottom of the microtube. Negative reactions may appear mixed-field when incompletely clotted serum samples are used in the gel test. Fibrin strands in such sera may trap unagglutinated RBCs, forming a thin line at the top of the gel. Other unagglutinated cells pass through the gel during centrifugation and travel to the bottom of the microtube. Before interpreting reactions as mixed-field, the clinical history of the patient should be considered. For example, recently transfused patients or bone marrow transplant recipients are expected to have mixed populations of RBCs, and their RBCs commonly produce mixed-field reactions.



■ FIGURE 15–5 Centrifugation of gel cards.

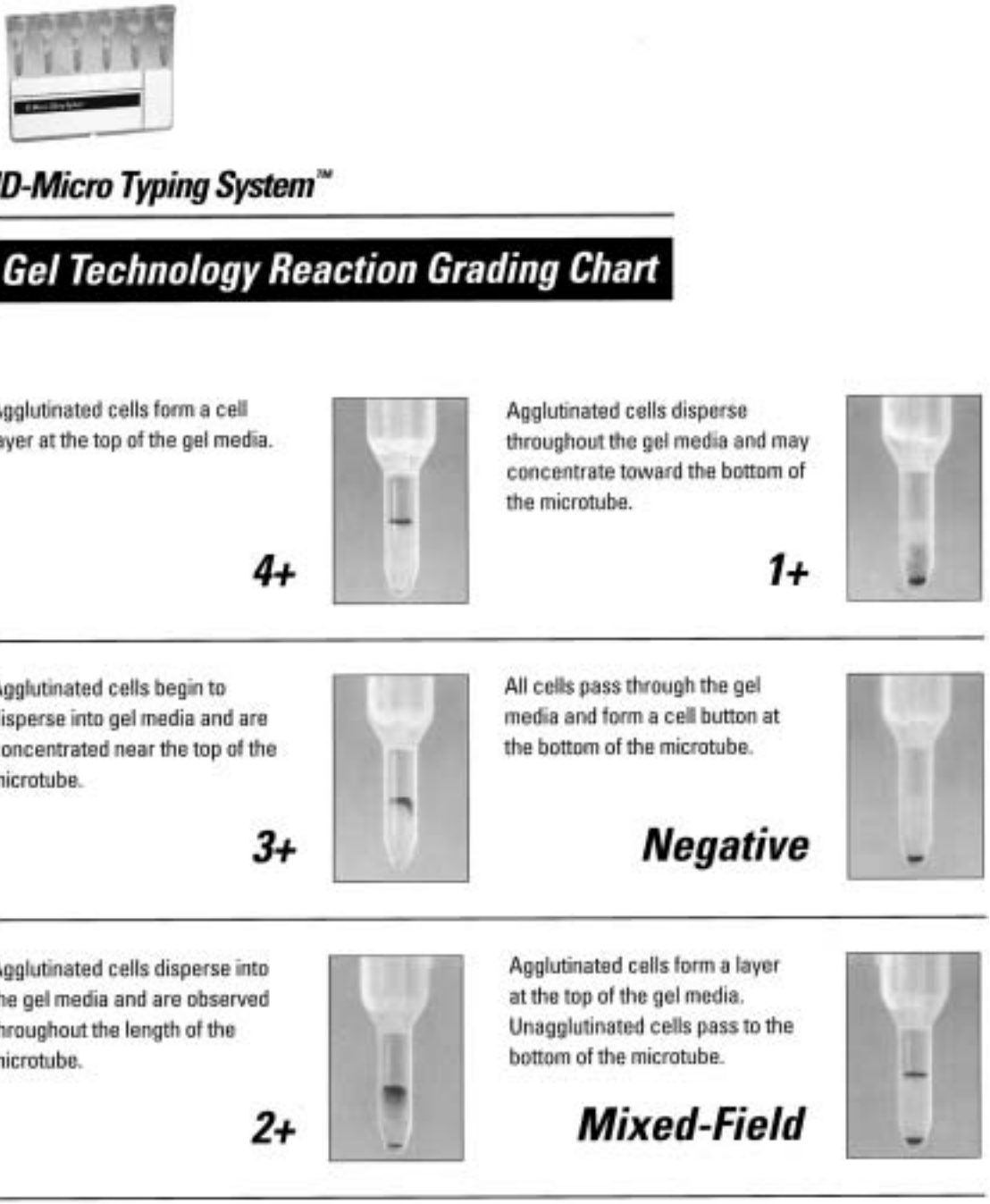
Tests Approved by the FDA

Gel technology is currently approved for ABO forward and reverse grouping, Rh typing, DAT, antibody screen, antibody identification, and compatibility testing. The ABO blood grouping card contains gels that include anti-A, anti-B, and anti-A,B for forward grouping. Microtubes with buffered gel are used for ABO reverse grouping.[3] The Rh typing card uses microtubes filled with gel containing anti-D.[4] The Rh phenotype card contains gels that contain anti-D, anti-C, anti-E, anti-c, anti-e, and a control.[5] Microtubes filled with gel containing anti-IgG are used for compatibility testing, antibody detection, and identification.[6]

Advantages and Disadvantages

Gel technology is applicable to a broad range of blood bank tests, and it offers several advantages over routine tube testing.[7] Standardization is one of the major advantages, inasmuch as there is no tube shaking to resuspend the RBC button. Tube shaking techniques vary among technologists, which results in variation in the reading, grading and interpretation of the test. The gel technique provides stable, well-defined endpoints of the agglutination reaction. It includes simple standardized procedures, no wash steps, and no need for antiglobulin control cells. These factors combine to produce more objective, consistent, and reproducible interpretation of the test results.

Because the gel technology offers objective, consistent results, it is ideally suited to individuals who have been cross-trained to work in the blood bank. Other advantages include the decreased sample volume needed for testing and the enhanced sensitivity and specificity of gel technology. Finally, gel technology offers improved productivity, standardization, and ability to meet regulatory requirements when compared with traditional tube testing.[8] **Table 15–1** describes these



■ **FIGURE 15–7** Gel technology reaction grading chart. Courtesy of Ortho Diagnostic Systems, Inc., Raritan, NJ, 1995

three advantages in more detail. The major disadvantage of the gel technology is the need to purchase special incubators and centrifuges to accommodate the microtube cards used for testing. In addition, a specific pipette must be used to dispense 25 µL of plasma or serum and 50 µL of a 0.8 percent suspension of RBCs into the reaction chambers of the microtubes.[9]

Solid-Phase Technology

History

Solid-phase immunoassays have been used for many years in immunology and chemistry laboratories. In these test systems (immunoassays), one of the test reactants (either antigen or antibody) is bound to a solid support (usually a microtiter well) before the test is started. The ability of plastics, such as polystyrene, to absorb proteins from solution and to bind them irreversibly made solid-phase serologic assays in plastic microplate wells possible. In 1978 Rosenfield and coworkers[10] were the first to apply the principle of solid-phase immunoassay to RBC typing and antibody screening tests. Other investigators were quick to follow with the solid-phase red cell adherence (SPRCA) technology. In 1984, Plapp and coworkers reported the use of SPRCA for the detection of RBC antigens and antibodies.[11,12]

SPRCA was developed commercially and manufactured

TABLE 15–1 Advantages of the Gel Technology in Terms of Productivity, Standardization, and Regulatory Issues

Improves Productivity	Increases Standardization	Addresses Regulatory Issues
Fewer procedural steps minimize hands-on time	Clearly defined endpoints promote uniform interpretation among technologists	Enhanced cGMP compliance attained through simplified training and standardized procedures
Standardized procedures simplify interpretation, reducing repeat and unnecessary testing	Use of precise measurements decreases test performance variability	Minimal handling of reagents and samples increases biosafety
Easy-to-perform testing enables optimal utilization of personnel	Elimination of technique-dependent steps improves consistency of test results	Test performance verification and NCCLS-formatted SOPs are provided to ensure easy implementation

cGMP = current good manufacturing practices; NCCLS = National Committee in Clinical Laboratory Standards; SOPs = standard operational procedures.

under the trade name of Capture® by Immucor for the detection of RBC- and platelet-related antibodies. In the Capture® technology, tests were adapted to microplate wells, either as full 96-well U-bottomed plates or as 1 × 8 or 2 × 8 strips of U-bottomed wells.[13] The first-generation SPRCA assays to be designed commercially included Capture-R® for the detection of RBC antibodies and Capture-P® for the detection of platelet antibodies.[13] To perform these tests, a laboratory centrifuge capable of holding 96-well microplates or strip wells was required, along with a microplate incubator and an illuminated reading surface or microplate reader **(Fig. 15–8)**.

Solid-phase immunoassays are currently available to detect antibodies to RBCs, platelets, syphilis, and cytomegalovirus.[14]

Principle

As mentioned previously, the principle of solid-phase immunoassay is based on SPRCA.11 The first-generation tests use chemically modified microplate test wells in which intact reagent RBCs are bound to the microwells before starting the test. Patient serum or plasma and low ionic strength saline (LISS) are added to the RBC-coated microwells and incubated at 37∞C. After incubation, the wells are washed free of residual serum proteins, an indicator of anti-IgG–coated RBCs is added, and the microwells are centrifuged. Centrifugation forces the indicator RBCs to contact the immobilized sensitized reagent RBCs. Positive tests show adherence of indicator RBCs to part or all of the well bottom, depending on the strength of the reaction **(Figs. 15–9** and **15–10)**.

In second-generation antibody screening tests, RBC membranes are bound to the microplate test wells and dried during the manufacturing process.

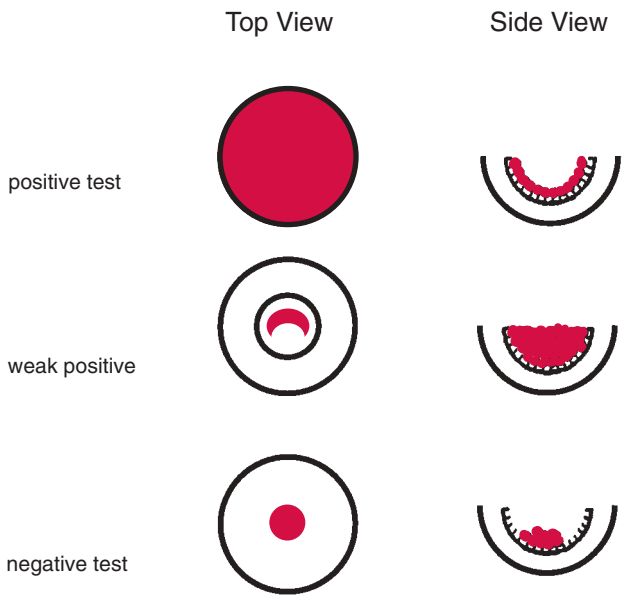
Capture-R® (Immucor) for the detection of RBC antibodies is a first-generation solid-phase test, and Capture-R® Ready-Screen/Capture-R® Ready-ID® (Immucor) are second-generation tests.[13]

Solidscreen® (Biotest) is a solid-phase test that detects antibody using microplate wells that are coated with polyspecific antihuman globulin (AHG).[13]

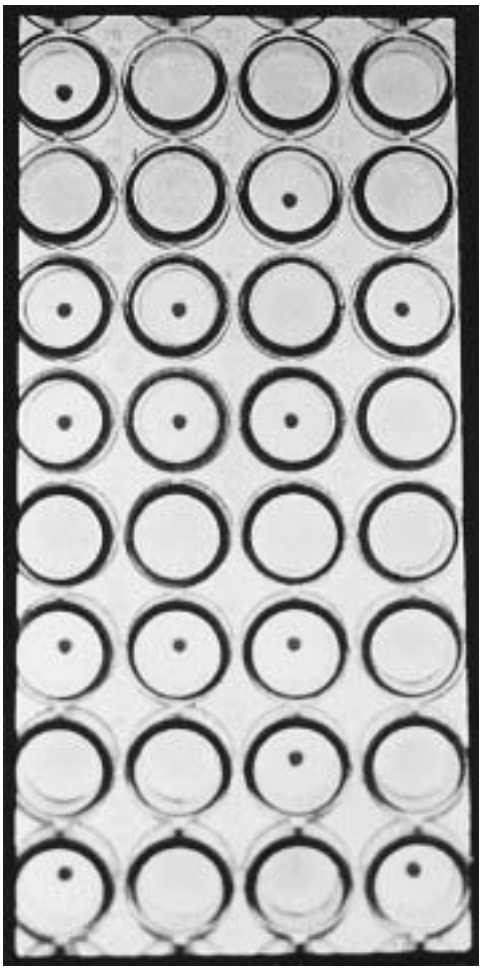
Test Reactions

In solid-phase technology, the target antigen (i.e., RBCs) is affixed to the bottom of the microplate wells. The test plasma or serum and LISS are added to the wells, and they are incu-

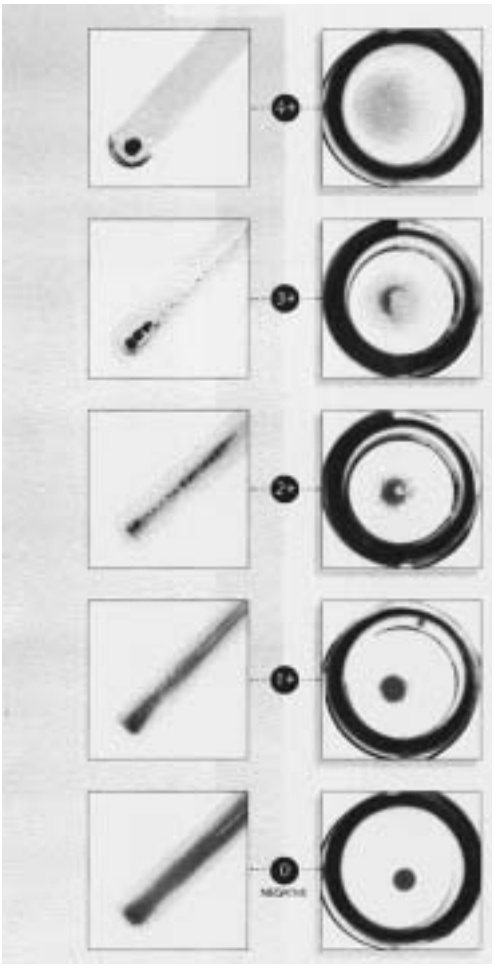
■ **FIGURE 15–8** Equipment for SPRCA technology.



■ **FIGURE 15–9** Test results: solid-phase cell adherence assay for the detection of antigens (illustration).



■ **FIGURE 15–10** Test results: solid-phase red cell adherence assay for the detection of antigens.



■ **FIGURE 15–11** Comparison of traditional tube test reactions with solid-phase reactions.

bated at 37°C to allow time for possible antibodies to attach to the antigen in the well. The wells are washed with pH-buffered isotonic saline to remove unbound plasma or serum; indicator cells are added; and the microplates are centrifuged. The indicator cells are AHG-coated RBCs. If antibody has attached to the antigen, the indicator cells will form a monolayer of RBCs. If no antibody is present, nothing is attached to the antigen, and the indicator cells form a clearly delineated button at the center of the microplate well following centrifugation. **Figure 15–11** compares test results from solid-phase technology with traditional tube testing reactions.

Solid-phase assays may be performed with either plasma or serum, but plasma is preferable. If a clotted sample is incompletely clotted, the serum is difficult to remove during the wash cycle. Residual unbound serum may clot and make the endpoint of the test unreadable. For best results, the manufacturer recommends adding a pH-stabilizing buffer to the isotonic saline that is used to wash the microplates. A suitable buffer is available from the manufacturer.[15]

Tests Approved by the FDA

Solid-phase technology is currently approved for antibody screening, antibody identification, and compatibility testing. Antibody screening cells are available as a two-cell (I and II) screen, four-cell screens, or as a pool of two cells. The two-cell screen is recommended for antibody detection for transfusion recipients. Pooled cells are used for donor antibody detection when increased sensitivity is undesirable. A panel is available for RBC antibody identification.

Advantages and Disadvantages

As with gel technology, standardization is the major advantage of solid-phase technology. Solid-phase technology provides stable, well-defined endpoints of the reaction. Objective, consistent, reproducible test results facilitate technologist training or cross-training. Other advantages include ease of use. No predilution of reagents is required. It is possible to test hemolyzed, lipemic, or icteric samples, and the enhanced sensitivity makes the detection of weak alloantibodies easier. The Immucor Capture® technology has the added safety feature that a color change in the LISS ensures that the patient sample was added to the test system.

The major disadvantage of solid-phase technology is the need for a centrifuge that can spin microplates, a 37°C incubator for microplates, and a light source for reading the final results. In addition, the increased sensitivity may also be a disadvantage inasmuch as solid-phase may detect weak autoantibodies that other systems miss.

TABLE 15–2 Current Tests Available for the Two Alternative Technologies*

Test	Gel Test (Ortho)	Solid-Phase (Immucor)
ABO—Forward	Yes	No
ABO—Reverse	Yes	No
Rh typing	Yes	No
Antibody screen	Yes	Yes
Crossmatch	Yes	Yes
Antibody identification	Yes	Yes
Auto-control (IgG only)	Yes	Yes
DAT	No	No

*Gel test. (Ortho Clinical Diagnostics, Inc., Raritan, NJ); solid-phase, Immucor, Inc., Norcross, GA.

Comparison of Technologies

This section compares the two alternative technologies in terms of equipment needed, procedures, test reactions, sensitivity, quality control, and automation.[16] Table 15–2 lists the current tests available for both of the alternative technologies, and Table 15–3 compares the features of the two technologies for routine blood bank testing.

Equipment

The gel technology requires special incubators and centrifuges to accommodate the cards. The solid-phase technology requires a centrifuge that can spin microplates, a 37°C incubator for microplates, and a light source for reading the final results. Automated and semiautomated equipment approved by the FDA is currently available for the solid-phase and gel technology.

Both technologies improve safety and decrease hazardous waste, inasmuch as the use of plastic eliminates the danger associated with broken glass, and miniaturized reaction chambers reduce the quantity of hazardous waste.

Test Reactions and Procedures

Both technologies demonstrate reproducible endpoints. The gel test uses a special pipette to precisely measure the quantity of test cells and sera. The solid-phase technology uses drops of cells and sera. By standardizing the reactants in the assay and eliminating variation in the tube-shaking technique, it is possible to eliminate the subjectivity associated with interpreting the endpoint of test tube agglutination tests. Quantitation of these assays is also less subjective than in conventional test tube technology. The gel test is read as 4+, 3+, 2+, 1+, MF or negative, and it is based on agglutination reactions. The ease of quantitating reactions with the gel test is an advantage when evaluating an antibody identification for multiple antibodies or dosage effects. Similarly, the solid-phase assays are read as weak positive, positive, or negative.

Mixed-field agglutination produces a characteristic pattern in the gel technology. In this technology, the agglutinated population of RBCs is trapped in the gel, and the unagglutinated population is pelleted at the bottom of the microtube following centrifugation. The ability to recognize mixed-field reactions is particularly valuable in evaluating a possible transfusion reaction or the survival of a minor population of transfused cells.

The endpoints of the new technologies are extremely stable, and they can be read 2 to 3 days after the test is performed. Such stability is a distinct advantage when less experienced technologists are cross-trained to work in the transfusion service. When the interpretation of an assay is unclear, it is helpful to be able to retain the test results until a supervisor can review them.

TABLE 15–3 Routine Blood Bank Testing: Comparison of Traditional and Alternative Methods

	Traditional	Gel	Solid-Phase
Reaction chamber	Tube	Microtube card	Microplate wells
Reaction patterns	Agglutination	Agglutination	Solid-phase immune adherence
Reaction matrix	None (cells and serum/plasma)	Immunologically inert dextran-acrylamide gels	Chemically modified polystyrene microplate wells
Testing detection	AHG (antihuman globulin sera)	AHG(antihuman globulin sera)	Anti-IgG–coated red cells
Washing required	Yes	No	Yes
Centrifugation required	Yes	Yes	Yes
Reaction readings	Quantitative: 1 + to 4+, MF	Quantitative: 1+ to 4+, MF	Semiquantitative: strong pos, pos, neg, no MF
Stable reactions	No	Yes (2–3 days)	Yes (2 days)
Quality control	Positive and negative controls/ Coombs' check cells	Lot number of cards and diluent on day of use	LISS color change, pos and neg control
Special equipment	No	Yes	Yes
Automation (FDA-approved)	No	Yes	Yes

MF = mixed-field.

The procedures for both technologies parallel steps performed in routine blood bank tube testing, including pipetting, incubating, spinning, and reading. The washing cycle step has been eliminated with the gel technology. Because the endpoint of both assays detects IgG, it is possible to perform an auto-control to detect IgG-coated cells. An auto-control cannot be substituted for a DAT, however, because none of the technologies currently detects C3d complement–coated cells. **Table 15–4** compares the procedural steps of the two technologies.

Sensitivity

Although both of the technologies are LISS-based, their sensitivity differ. The technologies have been shown to detect at least as many IgG antibodies as the conventional test tube methods using the LISS antiglobulin technique. The solid-phase technology is more sensitive than traditional serologic techniques. The sensitivity of all the technologies can be modified by pretreating the test cells or monolayer with enzymes, dithiothreitol, or chloroquine. Increased sensitivity is an advantage when a low-titered, clinically significant antibody is present, but it is a disadvantage when a low-titered warm autoantibody is present. Assays that detect only IgG avoid clinically insignificant IgM antibodies such as cold agglutinins; however, they also fail to detect clinically significant IgM antibodies that may be forming during a primary immune response. The alternative technologies detect IgG antibodies, including both clinically significant and insignificant IgG antibodies, such as high titer, low avidity antibodies. The gel technique may also detect IgM antibodies if incompatible cells form a lattice in the reaction chamber and are trapped at the top of the gel during centrifugation.

Quality Control

In addition to routine quality control (QC) for the 37°C incubator, centrifuge, pipette tips, and pipette dispenser used in testing, the new technologies have other QC features. The gel test uses special dispensers to prepare the RBC suspensions and special pipettes to add a measured volume of plasma/serum and RBCs. Each lot number of cards and diluent should be tested on the day of use to confirm that the test cards and the diluted reagent RBCs are reacting as expected. The manufacturer of the solid-phase technology recommends including a positive and a negative control with each batch of tests. In addition, in the solid-phase system, the LISS is formulated to detect the addition of plasma by a color change from purple to blue when plasma is added. This feature protects the user from failing to add plasma to a well.

Automation

Blood banks and transfusion services are the last areas of the clinical laboratory to move to automation. Chemistry, hematology, and immunology have been using automation for many years, but blood services have been hampered by the complexity of the testing and the subjectivity of the test interpretation. In recent years, new pressure has been applied to this area of the laboratory. Personnel shortages, turnaround time requirements, and the need for cost containment produced by increased managed care and greater regulatory demands have provided the incentive for blood services to seek automation. Automated equipment provides a partial solution to personnel shortages and turnaround requirements. By using walk-away automation, laboratory personnel are able to perform multiple tasks simultaneously. Automated equipment also provides the level of quality assurance required by new regulatory standards. Barcoding reduces identification errors by providing accurate patient and reagent identification. Standardized techniques reduce testing errors.

Automation in blood centers was first applied to infectious disease testing. These assays are easier to automate because they are less subjective than serologic tests. Although viral marker testing is performed in all blood centers, the equipment discussed in this chapter focuses on equipment that automates serologic testing in blood centers and transfusion services.

Gel Technology

Automated equipment for the gel test is being used successfully in Europe. Similar equipment (ProVue™) has received FDA approval in the United States. ProVue™ **(Fig. 15–12)** is a walk-away instrument with a capacity for 48 samples and 16 reagents. Instrument safety features include a bar-code tracking system and three cameras that record sample, reagent,

TABLE 15–4 Procedural Steps of Two Alternative Technologies

	Solid-Phase		
Procedural Steps	**Preloaded Microwells**	**Selected Cells**	**Gel Test**
1. Add RBCs to wells or tubes	N/A	Yes	Yes
2. Centrifuge plate to form monolayer	N/A	Yes	N/A
3. Wash away unbound RBCs	N/A	Yes	N/A
4. Add test serum/plasma	Yes	Yes	Yes
5. Incubation	15 min	15 min	15 min
6. Wash cycle	Yes	Yes	N/A
7. Add indicator cells	Yes	Yes	N/A
8. Centrifuge	2 min	2 min	10 min

* Solid-phase (Immucor, Inc., Norcross GA); gel test (Ortho Clinical Diagnostics, Inc., Raritan, NJ); RBC = red blood cells; N/A = not applicable.

■ FIGURE 15–12 ProVue™ instrument. (Reprinted with permission from Ortho Clinical Diagnostics, Inc.)

and card identification. A camera in the instrument performs image analysis and uses a mathematical algorithm to interpret the results. The gel test can be performed semi-automated using the TECAN MEGAFlex, a robotic liquid sample processor **(Fig. 15–13)**. The TECAN MEGAFlex instrument, which contains detectors for the liquid level and for clots, adds the reactants in the assay to the reaction chamber in the top of the microtube. Following incubation, the operator moves the gel cards into the MTS reader, which centrifuges and reads up to 24 cards at one time. A picture of each card's results, with strength of reactivity and result interpretation, is displayed on a computer screen. The operator reviews the display and accepts or changes the data, and then exports the data to the laboratory information system.

Solid-Phase Technology

Solid-phase technology has been fully automated, and two models of equipment are FDA-approved: the ABS2000 and the ROSYS Plato. The ABS2000 (see **Fig. 11–11**) is a walk-away instrument that performs ABO, Rh and donor confirmations by microplate hemagglutination technique, and IgG antibody screens and compatibility tests by solid-phase technique. The ROSYS Plato (see **Fig. 11–12**) is a semiautomated instrument that performs ABO and Rh testing by hemagglutination technique and screens for antibodies to RBC antigens, cytomegalovirus, and syphilis by solid phase technique. The ROSYS Plato has an off-line centrifuge and a stand-alone reader. The reader, the IBG Multireader Plus, reads hemagglutination and solid-phase results, interprets the results using an algorithm, and transmits the results to an information system. The ABS2000 and the ROSYS Plato have bar code readers to ensure proper identification of samples and reagents. In addition, these instruments have liquid level sensors, clot detectors, controlled temperature settings, and incubation timers.

■ FIGURE 15–13 TECAN MEGAFlex liquid handing system and MTS Reader M. (Reprinted with permission from Ortho Clinical Diagnostics, Inc.)

SUMMARY CHART:

Important Points to Remember (MT/MLT)

- The major advantages of these technologies over routine tube testing are:
- Standardization: There is no tube shaking or resuspension of an RBC button to cause subjectivity in the interpretation of the test.
- Stability: There are well-defined endpoints of the reaction.
- Decreased sample volume needed for testing
- Enhanced sensitivity and specificity
- The principle of the gel test is hemagglutination.
- In the gel test, RBCs and serum/plasma are allowed to incubate together in a reaction chamber.
- Following incubation, controlled centrifugation drives the RBCs through a specially designed microtube filled with beads of dextran-acrylamide gel.
- Agglutinated cells remain at the top of the tube or are trapped in the gel, depending on the size of the agglutinates.
- Unagglutinated cells move through the gel to the bottom of the tube.
- The gel test reactions are stable for observation or review for 2–3 days.
- Gel technology is currently approved for ABO forward and reverse grouping, Rh typing, DAT, antibody screening, antibody identification, and compatibility testing.
- The major disadvantage of the gel technology is the need to purchase special equipment: a centrifuge to accommodate the microtube cards used for testing and a pipettor and pipettes for dispensing plasma or serum and RBC suspensions into the reaction chambers of the microtubes.
- The principle of solid-phase technology is based on SPRCA.
- In solid-phase technology, the target antigen (RBCs) is affixed to the bottom of the microplate wells.
- If patient plasma contains antibodies to the antigen, the antibodies attach to the fixed antigen. Indicator cells detect attached antibodies by forming a monolayer of RBCs.
- If patient plasma contains no antibodies to the antigen, there is no attachment to the antigen, and the indicator cells form a clearly delineated button at the center of the microplate well.

- Solid-phase reactions are stable for observation or review for 2 days.
- Solid-phase technology is currently approved for antibody screening, antibody identification, and compatibility testing.
- Advantages of solid-phase technology include ease of use because no predilution of reagents is required and the ability to test hemolyzed, lipemic, or icteric samples. Enhanced sensitivity increases the detection of weak alloantibodies.
- The major disadvantage of solid-phase technology is the need to purchase special equipment: a centrifuge that can spin microplates, a 37°C incubator for microplates, and a light source for reading final results.

REVIEW QUESTIONS

1. The endpoint of the gel test is detected by:
   a. Agglutination
   b. Hemolysis
   c. Precipitation
   d. Attachment of indicator cells
2. The endpoint of the solid-phase test is detected by:
   a. Agglutination
   b. Hemolysis
   c. Precipitation
   d. Attachment of indicator cells
3. An advantage for both gel and solid-phase technology is:
   a. No cell washing steps
   b. Standardization
   c. Use of IgG-coated control cells
   d. Specialized equipment
4. A disadvantage for both gel and solid-phase technology is:
   a. Decreased sensitivity
   b. Inability to test hemolyzed, lipemic, or icteric samples
   c. Inability to detect C3d complement–coated cells
   d. Large sample requirement
5. A safety feature in the solid-phase test is:
   a. Air bubble barrier
   b. Viscous barrier
   c. Color change of the LISS
   d. Use of IgG-coated control cells

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