THE FUNDAMENTALS OF ...

Cell Washing Centrifuges

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Cell washing centrifuges are used in performing blood grouping, typing, compatibility and cross-matching; Rh and antiglobulin (AGT or Coombs) tests; and slide staining. They contain the same basic components as any other centrifuge: the electric motor, the rotor or head, the bowl, and the motor drive electronics. Some cell washing centrifuges also employ the cooling system used in "regular" refrigerated centrifuges. The characteristic that distinguishes a cell washing centrifuge is the special purpose rotor or head fitted to the motor shaft. This head facilitates automatic washing, decanting, mixing, and rewashing of red blood cells. Other ancillary components facilitate the concentration of cells onto slides for microscopic evaluation.

Many of the principles outlined in this article also apply to auto-transfusion units—medical devices that connect to a patient, collect specific components of their blood, and reinfuse the remainder plus replacement fluid. At the heart of most auto-transfusion units is a cell separating and washing centrifuge. In a sense, an auto-transfusion unit is the ultimate in semi or fully automatic control of a cell washing centrifuge and its ancillary components.

Current Technology

Cell washing begins as any other centrifuge operation: loading test tubes into their holders in the rotor. But unlike normal centrifugation, the caps are removed from the tubes before a lab technician places them into the rotor. Sometimes a separate cover is lowered and secured over the rotor, while other designs incorporate the cover into the lid. A typical wash cycle consists of filling the test tube with saline, centrifuging it, then removal of the supernatant. A subsequent cycle begins by resuspending the sediment in fresh saline solution, usually agitating the tubes, and then repeating the process.

Test tubes can be filled two ways: manually by the technician on basic models, or automatically through the use of a container of cell washing solution. That would normally be an IV bag of saline solution and a saline distribution manifold mounted in the cover or on the lid over the rotor on fully automatic models. Once the tubes are filled, they are generally agitated by rapid oscillating rotor movement and then centrifuged to separate the cells from the wash solution, which becomes supernatant. Once the rotor comes to a stop, the bottom of the tubes are held in place or tilted slightly outward, either electromagnetically or by deployment of a mechanical ring, to prevent them from swinging out during the next portion of the cycle. The rotor starts to revolve again at a lower speed, allowing the supernatant to be ejected from the top of the open test tube. Typically the ejected supernatant is caught by the internal cover, allowed to run down the sides of the internal cover, and collected in the bowl or internal trough of the centrifuge. It then exits the centrifuge where it either goes down the drain or is caught by an entrapment system and disposed of in accord-

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ance with local environmental laws. This basic cell washing process is used in a number of specialized centrifuges.

Cell washing centrifuges performing the direct antiglobulin test generally follow these basic steps using a 0.8% to 0.9% saline solution as the wash. The lab technician prepares a suspension of the patient's red blood cells in a 2% to 5% saline solution in several test tubes, places them in the centrifuge, and initiates the wash cycle. At the appropriate time, either the technician adds antiglobulin, or it is added automatically as part of the cycle. This mixture is agitated and recentrifuged one final time, and the automated cycle ends. The technician removes each tube from the centrifuge and examines the contents for agglutination of the red blood cells. If negative, the technician leaves the tube for about 10 minutes at room temperature, recentrifuges it, and reads it again. A positive reading allows for varying degrees of red blood cell agglutination.

Indirect antiblobulin tests are performed in a similar manner, except that the sample serum is first incubated with suspensions of several commercially available reagent red blood cells. The tubes are then placed into the cell washing centrifuge and processed similarly to the direct test. The lab tech then adds antiglobulin serum, recentrifuges the tubes, and checks for agglutination.

The cytological centrifuge is an adaptation of the basic cell washing variety, and automatically prepares slides for microscopic examination. The cytological centrifuge uses the outward force generated by the rotation of the head to place cells suspended in a fluid onto microscopic slides. Typically, the lab technician fits together a specimen container, disposable filter card or absorption pad containing two round holes, and a slide. The technician then fills the specimen container. One such assembly is prepared for each slide, and each assembly is placed into a special centrifuge head. The rotor holds the fluid container at an angle away from the filter card while at rest. When rotated, the container tips, driving the fluid into contact with the filter card and glass slide. The outward force of the rotating head creates a layer of cells on one or two small areas of the glass slide over the holes in the filter card. The remainder of the fluid is absorbed by the filter card or absorption pad. The centrifuged slide is then removed from the rotor and fixed using prescribed procedures. If the specimen container is designed for a single use, the entire assembly is discarded. If the specimen container is reusable, the chamber is cleaned and dried; only the filter card and absorption pad are discarded.

Origin and Evolution

Cell washing centrifuges do not have a long, illustrious, or well-documented history. In fact, the earliest patents on these devices were only granted in the late 1960s. Most of them resulted from the adaptation of conventional centrifuge designs by laboratory tinkers, and represented incremental improvements to the manual processing methods. Eventually, they resulted in the current designs of cell washing centrifuges we find today.

How to Manage the Device

The rules and management techniques used for general purpose centrifuges apply to the cell washing type as well. It's a good idea for the laboratory to maintain copies of both preventive and remedial service records, as prescribed by its accreditation organization.

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Regulations

As is the case with general purpose centrifuges, the College of American Pathologists (CAP), the American Association of Blood Banks (AABB), and The Joint Commission (TJC) certify laboratories and healthcare facilities that employ cell washing centrifuges. Although not regulatory agencies, their certification is necessary for the receipt of federal funding—particularly from Medicare and Medicaid—and seen as a mark of competency, quality, and accuracy. Private sector insurance companies typically follow the lead of the federal government in determining rules for paying providers. Therefore, certification is a virtual requirement for all nonpersonal payments. Maintenance procedures are outlined in the publications available from the various certifying bodies.

Risk Management Issues

Of the risks found in general purpose centrifuges, the cell washing type are particularly sensitive to rotational speed, timing, and braking issues. These are key parameters for ensuring proper processing of the cells. The correct speed and timing ensures proper separation between cells and the wash solution.

Braking effectiveness ensures the head has come to a complete stop, which is especially important after decanting, before another wash cycle begins. Improper washing and decanting of the supernatant will adversely affect the end product and distort the final results.

Additionally, the cell washing centrifuge is designed for only one size of test tube, typically either 10 X 75 mm or 12 X 75 mm. Likewise, the system is designed to use a specific inside diameter of plastic tubing for dispensing the rinsing saline. Using a different inside diameter will change the volume of saline dispensed during the timed filling period. The use of other sizes of test tubes and tubing must be avoided since this will also distort the final results. Lastly, improperly cleaned sample chambers can result in cross-contamination between patient samples and result in false readings.

Troubleshooting

To minimize risks, regular and proper scheduled maintenance of cell washing centrifuges is absolutely essential. Additionally, automatic braking must be included in regular preventive maintenance checks to ensure it stops rotating within the allotted time to maintain cycle sequencing. Fortunately, today's centrifuges are relatively maintenance and failure free. As with general purpose centrifuges, their main problem areas are brushes and bearings in the electric motor. Most modern units employ brushless induction motors and long-wearing bearings to minimize their problems.

Training and Equipment

Since a typical cell washing centrifuge is an adaptation of its general purpose cousin in the manufacturer's product line, the training and equipment necessary to service the base—consisting primarily of the motor and control electronics—is the same. The two most important service aids are a tachometer, to determine and confirm that the centrifuge is operating at the correct speed, and a stopwatch to confirm the accuracy of cycle times. The upper portion or bowl components—the rotor, saline distribution manifold, mechanism to hold the bottom of the tubes in place, internal cover or centrifuge lid, the bowl of the centrifuge, and drain—are straightforward items. Likewise, supporting external items such as a saline pump and solenoid valves are generally standard and off the shelf. Manufacturer's literature coupled with common hand tools should take care of most problems with cell washing centrifuges. Manufacturer training would be a plus, although it is not mandatory to service these devices.

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Future Development

Cell washing centrifuges employ induction motors, permanently lubricated bearings, and microprocessor technology to control their operating cycles. They are the midpoint between completely manual and fully automated cell processing. Additional improvements on the horizon include multiuse cell washing centrifuges that automatically "sense" which rotor is installed by reading a bar code and accessing an on-board database to set up the processing parameters. ■

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Glossary of Terms

Agglutination: the process by which suspended bacteria, cells, or other particles are caused to adhere and form into clumps; similar to precipitation, but the particles are larger and are in suspension rather than being in solution.

Antiglobulin: an antibody directed against gamma globulin.

Decant: to pour off (wine, for example) without disturbing the sediment or to pour a liquid from one container to another.

Supernatant: the soluble liquid portion of a sample after centrifugation or precipitation of insoluble solid matter.