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Hemagglutination Assay and Inhibition Test: A Tool for Viral and Bacterial Reaction The presence of antigen-antibody reaction can be observed in red blood cell (RBC) clumping, which is visible to the naked eye or under a microscope. Agglutination occurs when the antigens and their associated antibodies are present and interact. A negative test result is indicated by the absence of clumping. The Hemagglutination Assay uses RBC suspension as a carrier particle coated with specific antigens or antibodies. The assay can use various RBC types from animals such as humans, sheep, chicks, etc., to detect specific antigens. Positive control samples contain known concentrations of target materials to validate assay sensitivity and specificity. Negative control samples do not contain the target substance but serve as a baseline for comparison. Microtitre plates are commonly used in hemagglutination experiments due to their ideal environment for reaction between RBCs and target substances. PBS maintains pH and ionic balance required for effective hemagglutination. Rapid hemagglutination assay kits can be used for quick testing, comprising slides, reagents, and control samples designed for ease of use. The Hemagglutination Assay is useful in determining hemagglutinins in allantoic fluid from embryonated eggs, offering advantages over the Rapid Diagnosis Test. The Micro-hemagglutination Assay provides a standardized cell suspension using dissolved red blood cells in a 1% solution, with V-bottom plates recommended for efficient settling and settling pattern examination. This assay offers improved precision and accuracy due to distinct positive and negative results, making it beneficial in research settings where detailed hemagglutinin analysis is required. Negative and positive control samples, along with inoculated eggs, are required for the procedure. The eggs should be chilled for at least 2 hours or overnight before use. To avoid contamination, it is crucial to change pipette tips between each sample. Begin by recording relevant details about the samples on a sheet. This will aid in tracking and recording assay results. Take approximately 50 µl of each sample with a micropipette and dispense it into a specified well of the microwell plate. Use new pipette tips for each sample to prevent contamination. Place negative and positive controls on one of the plates. Add 50 µl of Phosphate Buffered Saline (PBS) to each well, then add 25 µl of 1% red blood cells. Gently tap the plate's sides to mix the contents of each well, allowing samples to interact with the red blood cells. Cover the plate and allow it to stand for around 40 minutes to enable the reaction. Observe and record data during this incubation period. Note any obvious agglutination or absence of agglutination in each well. Red blood cells settle at the edges of the V-shaped well, forming a sharp button at the bottom, indicating a negative HA result. If agglutination occurs, red blood cells do not form a distinct button but instead create a diffuse film, signifying a positive result for the specific antigen or antibody being tested. A control using only red blood cells is provided as part of the test. Individual red blood cells should settle at the edges of the control wells, forming a sharp button identical to the negative HA result. This ensures that red blood cells are functioning properly and serves as a baseline against which test results can be compared. Note: The HA titer of two samples is represented in Figure 2, with agglutination indicating a positive result and dots or beads representing negative results (Sharma et al.). To optimize the hemagglutination test, it is sufficient to evaluate only one set of negative and positive control samples. This involves analyzing the settling pattern of the red blood cell suspension during the test. To achieve this: Fill the designated area with diluent and gently mix the red blood cells with the diluent. Allow the mixture to settle for a period of time. Examine the settled cell pattern, ensuring it follows a typical pattern with no signs of auto-agglutination or clumping. Before initiating the test, ensure that all kit components are at room temperature (15-30°C) and allow them to equilibrate for approximately 30 minutes. Mix the liquid reagents gently and calculate the total number of samples to be tested as well as the required plate size. Label each well with the last three numbers of the donor's ID, and arrange the samples in a sampling rack according to the plate map. Add 190 µl of sample diluent to the first additional well, followed by 10 µl of specimen with 190 µl of sample diluent using a fresh pipette tip for each addition. Fill each control and test well with 25 µl of the diluted sample, then add one drop of suspension (about 75 µl) to each well, including the positive and negative control wells. Mix the contents of each well by slowly rotating the plate in a circular motion, maintaining a level and stable surface. Incubate the plate for one hour at room temperature, then examine the data after incubation. Check each well for the presence or absence of agglutination, with a positive result indicating agglutination and a negative result indicating its absence. Note that if any control well fails to produce a distinct button-like structure, this may indicate a technical issue, requiring the test to be repeated to ensure accurate results. Test cells indicate the presence of anti-cell antibodies; however, in this scenario, the test is rendered invalid, necessitating serum absorption. To rectify this situation, 1/4 of the test serum is diluted with control cells and left at room temperature for 5 minutes following centrifugation at 1000 rpm. The resulting supernatant is then further diluted by one-fifth with the diluent. This diluted material can be tested directly using suspensions of test and control cells without additional dilution. A nonreactive result, marked by a compact button in the absence of agglutination, suggests the absence of the target substance being tested for. Conversely, a reactive result is characterized by a typical ring pattern or net formation in the test well, whereas the control well exhibits a compact button formation. Reiterating, positive TPHA test results must be retested to validate findings; if consistent, it becomes critical to isolate and identify the sample. Additional assays, such as RPR, can aid in determining infection existence or antibody presence. The hemagglutination assay provides valuable insights into humoral immune responses against infections and detect certain antibodies in patient serum. This technique has been instrumental in creating rapid diagnostic test kits that leverage hemagglutination-based responses to quickly detect specific diseases or antigens. For example, hemagglutination is crucial in RDT kits designed to detect Hepatitis B surface antigen. The assay also plays a pivotal role in detecting and quantifying viral infections, particularly those with hemagglutination characteristics such as paramyxovirus and influenza. Furthermore, the hemagglutination assay facilitates diagnosis and monitoring of viral infections by evaluating agglutination reactions between viruses and red blood cells. This technique is also employed to detect certain bacterial infections, like syphilis, and to distinguish blood cell types or groupings by identifying specific antigens on red blood cells. Various laboratory equipment, including microtitre plates, pipettes, and centrifuges, are commonly utilized in hemagglutination assays due to their cost-effectiveness and minimal specificity for a particular test. These equipment enables rapid interpretation of data in standardized formats, such as the Rapid Diagnostic Test (RDT), which aims to produce quick and easily readable results within a limited timeframe. The hemagglutination assay is a helpful tool in point-of-care settings or situations where quick decision-making is necessary for further diagnosis due to its simplicity and ease of execution. It involves mixing samples, red blood cells, and specific reagents followed by evaluating the agglutination reaction. The straightforward protocol makes it easier to implement and reduces the possibility of procedural errors. This test is often less expensive than other diagnostic tests because the required chemicals and equipment are frequently inexpensive and widely available. However, interpretation of results may vary slightly among observers, which can lead to conflicts or inaccuracies, but establishing clear interpretation standards and providing frequent training can mitigate this issue. The hemagglutination assay's sensitivity to experimental factors like incubation time and RBC concentration requires careful attention to produce reliable and reproducible results. Additionally, the determination of quantitative values and interpretation of results may necessitate the use of experienced professionals familiar with the assay principles and applicable standards to ensure precise and trustworthy analysis. Various methods exist for detecting influenza viruses, including the Haemagglutination Inhibition Test (HAI). This test takes advantage of a virus's ability to cause red blood cells from mammals or birds to clump together. Viruses such as influenza, parainfluenza, and adenoviruses can all induce haemagglutination in different species' red blood cells. The HAI test relies on antibodies that target the viral protein responsible for this agglutination, thereby preventing it from occurring. The specificity of the HAI test varies depending on the virus being tested. For example, with influenza A viruses, the antigen responsible for haemagglutination is the same as the one involved in virus adsorption and neutralization, making the HAI test very specific. However, other viruses like flaviviruses may produce cross-reactive antibodies that can interfere with the test's results. The HAI test is generally more sensitive than complement-fixation tests but less so than Enzyme Immunoassays (EIAs) or Radioimmunoassays (RIAs). It requires minimal equipment and is cost-effective, making it a popular choice for diagnosing rubella and influenza infections. To perform the HAI test, patient sera are serially diluted and allowed to react with a fixed amount of viral haemagglutinin, followed by the addition of red blood cells that can be agglutinated. However, the presence of non-specific inhibitors of viral haemagglutination or naturally occurring agglutinins in the erythrocytes may complicate the test. To minimize this issue, sera should be treated before use to remove any potential inhibitors. The HAI test is widely used for diagnosing rubella and influenza virus infections, with specific protocols varying depending on the virus being tested. For example, when testing for rubella, one-day-old chick or goose erythrocytes are used, along with bovine albumin veronal buffer as a diluent. A fixed amount of 4 haemagglutination units of rubella antigen is required, and the actual concentration should be determined before each test by titrating the antigen from 1:2 to 1:1024. The patients' sera are then diluted in BAVB from 1:8 to 1:1024, using either V-shaped or U-shaped microtitre plates. To remove non-specific inhibitors of viral haemagglutination, sera can be treated with kaolin, RDE, potassium periodate (KIO), or heat inactivation. Non-specific agglutinins for erythrocytes may also be removed by adding erythrocytes to the sera before testing, allowing the erythrocytes to absorb any non-specific agglutinins present. Only a previous HAI test has produced similar results. The procedure begins with the addition of 4HA of rubella antigen to each well containing diluted test sera, excluding serum control wells. A back titration of rubella antigen should span 4 HA units to 0.25 HA units. After 60 minutes at room temperature, either 0.5% goose cells or 0.4% chick cells are added to each well and incubated at 40C for 60 minutes before reading the plate. Serum controls should display no agglutination, while erythrocytes-only control wells should exhibit a button at the bottom of the well. The haemagglutinin back titration should show agglutination at 4, 2, and 1 HA units. A fourfold or greater rise in HAI antibody between acute and convalescent phase sera indicates recent rubella infection. HAI tests are relatively easy and inexpensive to perform, but they are less sensitive than EIAs or RIAs, with subjective result reading and the need for fresh reagents to avoid abnormal agglutination patterns that complicate test interpretation. As a result, the HAI test has been largely replaced by more reliable EIA and RIA tests for rubella IgG in many diagnostic laboratories.

Hemagglutination inhibition test results. Hemagglutination inhibition test positive result. Hemagglutination inhibition test principle. Hemagglutination-inhibition tests.