



Structure, Mechanism, and Principle of Immunochromatography for Antigen Detection

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DESCRIPTION

The lateral flow test, or Immunochromatography Assay (ICA), is a simple device used to detect the presence or absence of a target analyte. Immunochromatography combines chromatography (the separation of components of a sample based on changes in how they pass through a sorbent) with immunochemical reactions. The test strip is the most widely used immunochromatographic system. Antigens (for antibody detection) or antibodies (for antigen detection) are immobilised on a nitrocellulose membrane in discrete areas in a cartridge device, depending on the test. The consecutive addition of an enzyme-labeled conjugate and substrate results in the development of a coloured reaction product immediately on the membrane after antibody or antigen capture and a flow-through wash step.

Main components of immunochromatographic test strip

Sample application pad: The sample is put on this pad, which is constructed of cellulose and/or glass fibre, to begin the assay. Its job is to transport the sample to other parts of the system. The sample pad should be able to transport the sample in a smooth, consistent, and uniform manner. This preparation may comprise sample component separation, interference removal, pH adjustment, and other procedures. To begin the test, place the analyte sample in the sample application pad.

Conjugate pad: Labeled biorecognition molecules (labelled antibodies, mainly nanocolloid gold particles) are administered here. When the conjugate pad comes into contact with a moving liquid sample, the material should rapidly release the labelled conjugate. The labelled conjugate should remain stable during the lateral flow strip's lifetime. Variations in conjugate dispensing, drying, or release can drastically alter assay findings. The sensitivity of the assay can be affected by improper labelled conjugate production. Conjugate pads are made of glass fibre, cellulose, polyester, and other materials.

Substrate (nitrocellulose) membrane: It is quite important in assessing ICA sensitivity. Over this piece of membrane, test and control lines are drawn. As a result, an ideal membrane should provide support as well as good probe binding (antibodies, etc.). Nonspecific adsorption over test and control lines can considerably alter assay findings. Hence, a good membrane will have less non-specific adsorption in the test and control line regions. The assay's sensitivity is improved by proper bio-reagent dispensing, drying, and blocking.

Adsorbent pad: At the end of the strip, it serves as a sink. It also aids in controlling the liquid's flow rate across the membrane and prevents sample backflow. The ability of the adsorbent to hold liquid can have a significant impact on the assay findings. All of these elements are attached to or mounted on a background card. Because the backing card has nothing to do with ICA other than serve as a foundation for the proper assembly of all the components, the materials used for it are extremely adaptable. As a result, the backing card acts as a support and makes handling the strip much easier.

Principle for the detection of antigen

In clinical microbiology laboratories, lateral flow immunoassays are mainly double-antibody sandwich tests. The capture zone (test line) on the membrane contains immobilised antibodies for antigen detection. The specimen containing the antigen to be identified (e.g., serum, urine) is placed on the sample pad, which absorbs the specimen fluid. The fluid subsequently migrates to the conjugate pad, which includes antigen-specific conjugated antibodies (gold, coloured latex, or chromophore conjugated). The antigen-antibody-conjugate complex is generated in this step. The Ag-Ab complex travels across the membrane until it reaches the capture zone, where it binds to immobilised antibodies. The "test" line becomes apparent on the membrane as more Ag-Ab complexes are trapped there. The sample then migrates further down the strip until it reaches the control zone, where extra conjugates attach to the membrane and form a

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Received: 03-May-2022, Manuscript No. JFB-22-16775; **Editor assigned:** 06-May-2022, Pre QC No. JFB-22-16775 (PQ); **Reviewed:** 20-May-2022, QC No. JFB-22-16775; **Revised:** 27-May-2022, Manuscript No. JFB-22-16775 (R); **Published:** 03-Jun-2022, DOI: 10.35248/2090-2697.22.13.398.

Citation: Waters K (2022) Structure, Mechanism, and Principle of Immunochromatography for Antigen Detection. J Forensic Biomech.13:398.

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second visible line (the control line). This control line shows that the sample has properly migrated over the membrane.

Antibodies that aren't antigen-specific or conjugated antibodies that aren't complex with antigen are not collected in the test line and go on to the control line. The control line is made up of immobilised anti-immunoglobulin antibodies. Uncomplexed antibodies are caught and become evident at the control line as more and more uncomplexed antibodies pass over it. The inclusion of a control line only means that the test was completed successfully. Test result interpretations are as follows: A clean line in the control zone and the test area on the membrane indicates a positive result. A single line in the control zone indicates a negative outcome. A single line in the test region with no corresponding control line is indicated as invalid.

CONCLUSION

Toxin detection, pregnancy tests, detection of Human Chorionic Gonadotropin (hCG), diagnosis of parasitic diseases, diagnosis of bacterial infections, and diagnosis of viral infections are all common uses of immunochromatographic methods in clinical practise. Immunochromatographic methods have the various advantages of commercial availability and low cost (in comparison to EIA, Immunofluorescence, or RIA), comparable or better sensitivity and specificity than other well-established methods, rapid test, small sample volume requirement, easy to perform (no sample pre-treatment required in most cases), simple and user-friendly (to perform and interpret test results), and can be used in the field. The limitations are it is largely qualitative or semi-quantitative, and most instruments can detect many analytes at the same time.