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# Dot Blots Analysis in the Separation of Anti-HIV Antibodies.

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## ABSTRACT

Dot blot frequently is underutilized or is not frequently used because it cannot assess the molecular weight of macromolecules, but it is simple to perform and is useful when a particular biomolecule needs to be identified. In this protocol dot blot analysis is used for detection of anti-HIV antibody [1], and it is also available for determination or separation of any protein. It can be very useful in the immunodiagnosis of infectious diseases caused by viruses [1] and bacteria [2] and in addition for the determination of specific DNA sequences by using probes [3].

## References

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## MATERIALS

NAME	CATALOG #	VENDOR
3,3',5,5'-Tetramethylbenzidine	54827-17-7	Sigma Aldrich
Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum 500 mL	7905	Stemcell Technologies
Nitrocellulose Membrane, Precut, 0.45 µm, 7 x 8.5 cm	1620145	BioRad Sciences
BioDot SF apparatus (Bio-Rad Laboratories Richmond CA USA).		
Peroxidase-labeled HIV proteins; Murex Diagnostics Norcross USA)		

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- 1 Two (2) µl of 1:2 dilutions of chicken IgY or 2 µl of serum from rats and cats is dotted onto nitrocellulose paper. This animal specimens tested positive for the presence of anti-HIV antibodies by ELISA, after being immunized with HIV proteins.
- 2 Place the nitrocellulose membrane in a BioDot SF apparatus (Bio-Rad Laboratories, Richmond, CA, USA).
- 3 The membrane is blocked with 5 µL/well of fetal bovine serum with 1%Tris buffer saline.
- 4 Then 5 µL of a commercial conjugate (peroxidase-labeled HIV proteins; Murex Diagnostics, Norcross, USA) is added and allow to drain by gravity.
- 5 Finally, 5 µL of the substrate 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co., St Louis, MO, USA) is added and the mixture is incubated for 20 min.
- 6 The reaction is stopped by washing the wells with distilled water under a vacuum.
- 7 The membrane is left to dry.
- 8 Visualization of color development.

