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A Quick Look at Immunofluorescence

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NOTES AND COMMENTS:

A Quick Look at Immunofluorescence

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Immunofluorescence microscopy is an advanced laboratory technic having diverse clinical diagnostic applicability. Its principle, stated most simply, is that fluorochrome-labeled antibody can be followed in an immune complex exposed to ultraviolet light. The method has specificity equal to the combining quality of the particular antibody, and it ranks among the most sensitive immunologic methods. Reliability is usually maximal when the technic is performed by an experienced operator.

One of three basic methods can be employed: Direct immunoflourescence entails reaction of labeled antibody upon its homologous antigen (Figure1). The indirect method (Figure 2) involves a two-step procedure in which unlabeled antibody combines with antigen, followed by reaction of the aggregate thus formed with a labeled anti-antibody. Sensitivity is thereby enhanced over the direct method because more surface reactive sites are available to the labeled antibody. The anticomplement system, diagrammed in Figure 3 is based upon the avidity of serum complement for many antigen-antibody complexes. Labeled anticomplement antibody (anti B_{1C} - B_{1A} globulins) is employed to demonstrate the complex formed by original antigen, antibody and complement. Sensitivity equals that of the indirect method.[1]

Schematic representation of the fluorescent microscope's optical path and filter components may be seen in Figure 4.

The direct method permits rapid identification of microorganisms in clinical specimens such as naso-pharyngeal swabs or spinal fluids (Figures 5 and 6), but type-selective conjugated sera are required for each organism.[2]

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DIRECT FLUORESCENT ANTIBODY METHOD

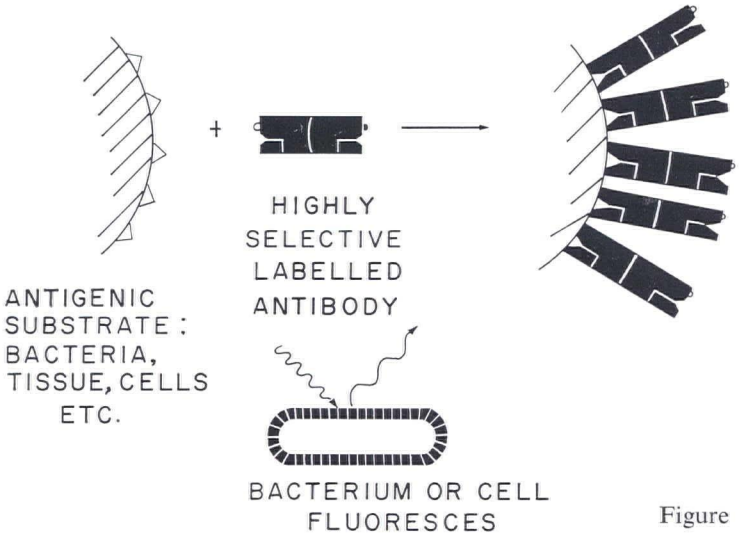

Figure 1

INDIRECT FLUORESCENT ANTIBODY METHOD

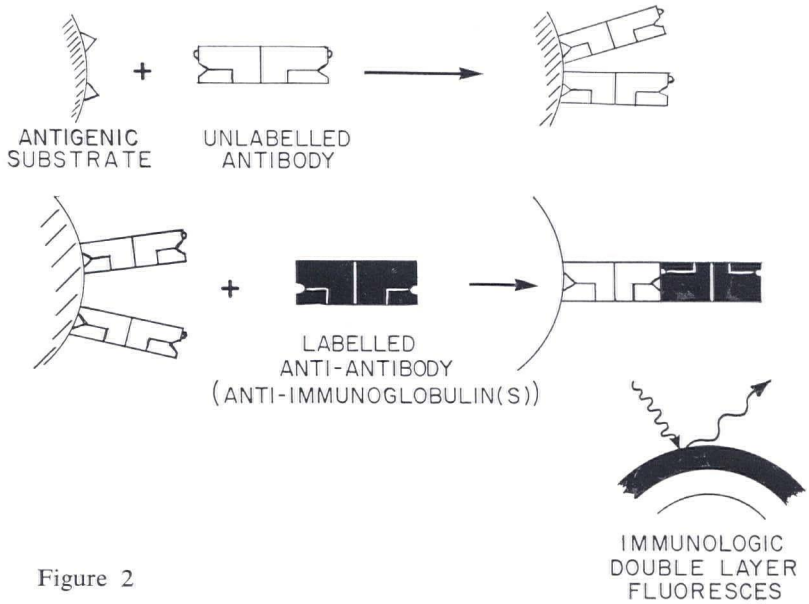

Figure 2

A Quick Look at Immunofluorescence

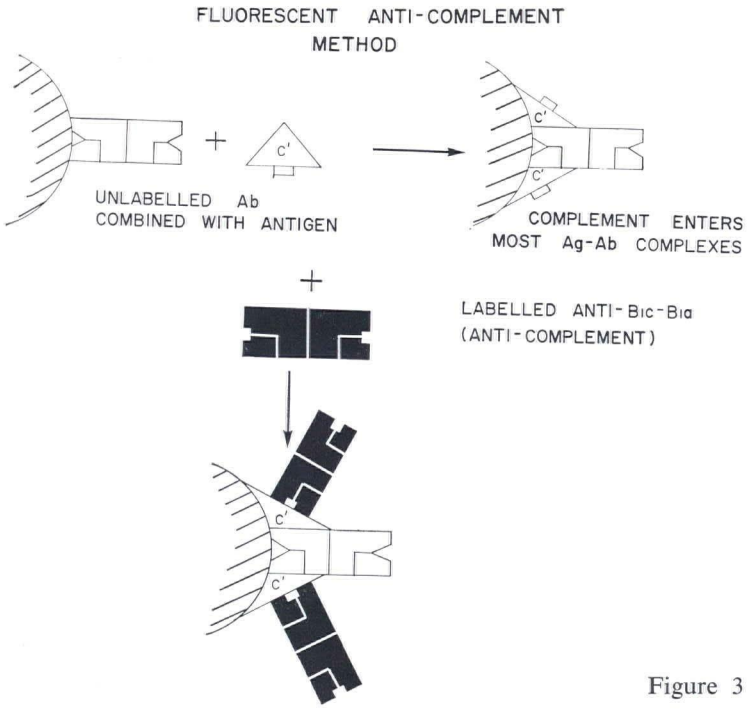

Figure 3

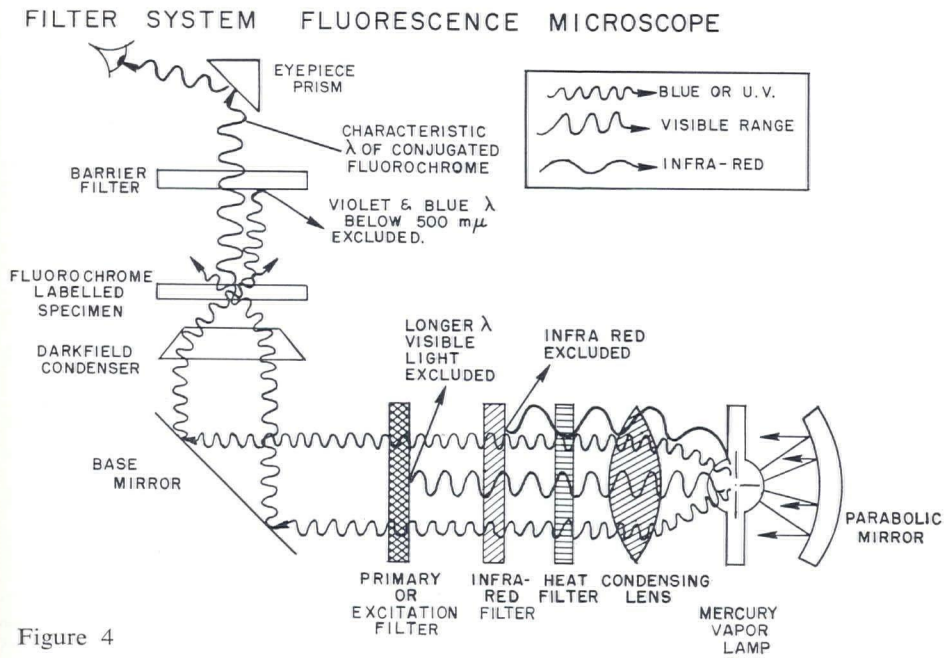

Figure 4

The FTA-ABS test for syphilis (Figure 7) utilizes the indirect method to detect patient antibody against Nichol's *Treponema pallidum* using an intact, killed spirochetal test antigen. The method is sensitive, highly selective and has nearly eliminated false positivity.[3]

Antinuclear antibodies, valuable in the diagnosis of connective tissue diseases, are demonstrable (Figure 8) via the indirect technic upon nuclear substrate provided by tumor cell imprints. A negative reaction essentially rules out active systemic lupus erythematosus.[4]

Localized gamma globulin detectable at the dermal-epidermal junction (Figure 9) in involved skin biopsy sections is presumptive evidence of *in vivo* antibody localization and confirms a diagnosis of L.E.[5]

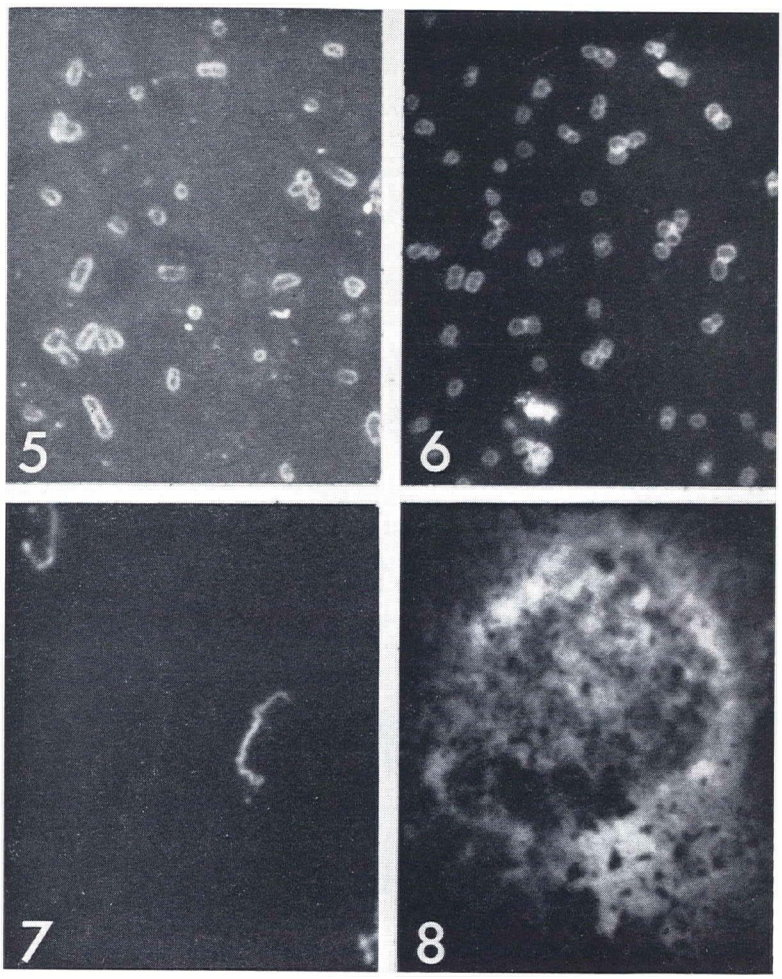

Figure 5: Haemophilus influenzae; figure 6: Neisseria meningiditis (note paired cells); figure 7: Treponema pallidum (Nichol's strain); figure 8: "Thready" type. Nuclear immunofluorescence upon a leiomyosarcoma imprint.

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Globulin localized in kidney biopsy sections (Figure 10) can be visualized via the indirect or anticomplement immunofluorescence microscopic methods and is, like the fluorescent skin band, presumptively, bound antibody. Clinical significance of such kidney-bound globulin is currently under investigation at this institution.

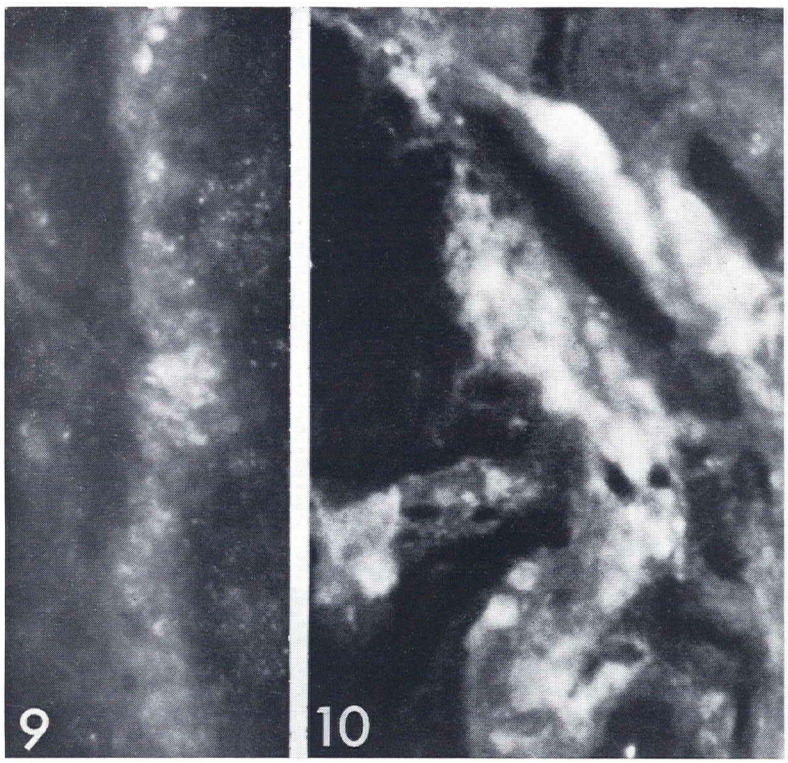

Figure 9: Immunofluorescent skin band, dermal-epidermal junction; figure 10: Localized IgG-kidney biopsy section.

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*all photographs x 950, Leitz fluorite oil immersion objective