NOVA Lite® Monkey Ovary, Testis, Adrenal IFA Slides
For In Vitro Diagnostic Use
For Export Only. Not for sale in the United States.

Product Code: 504220, 504220.10

CLIA Complexity: High

Intended Use
Monkey ovary, testis, adrenal sections are intended for use in indirect immunofluorescence assays (IFA), screening human serum for circulating antibodies to steroid cells rather than using separate ovary, testis and adrenal slides.

Summary and Explanation of the test
Steroid cell autoantibodies bind to cytoplasmic antigens of steroid producing cells in testicular Leydig cells, ovarian thecal cells as well as placenta and adrenal cortex. These IgG antibodies are present in patients with Addison's disease and in some patients with ovarian or testicular failure and/or associated hypoparathyroidism.\(^1\)\(^-\)\(^3\)

Principles of the Procedure
These slides are for use in an indirect immunofluorescence technique where patient samples and appropriate controls are incubated with the sections. The unreacted antibodies are washed off and then appropriate fluorescein-labeled conjugates are applied. Unbound conjugate is washed off as before. Slides are viewed with a fluorescence microscope and positive samples produce apple-green fluorescence that corresponds to areas of the section where autoantibody has bound.\(^4\)

Reagents
Monkey ovary, testis, adrenal sections on 5-well slides individually wrapped in a foil pouch containing a desiccant

Warnings/Precautions
Proper handling and disposal methods should be established for all potentially infective samples tested with this product; only personnel adequately trained in such methods should be permitted to perform the procedures.

Storage Conditions
Unopened slides should be stored at 2-8°C and can be used until the given expiry date. DO NOT FREEZE. Once slides are removed from a foil bag, they should be used immediately.

Specimen Collection
Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 7 days prior to assay\(^6\), or for prolonged storage, aliquoted and stored at -20°C or below. DO NOT freeze and thaw sera more than once. Avoid using lipaemic, haemolysed or microbially contaminated sera as decreased titers or unclear staining patterns may occur.

Procedure
Materials provided
1. Monkey Ovary Testis Adrenal – 1 x 5-well slide or Monkey Ovary Testis Adrenal 10 x 5-well slides

Additional Materials Required But Not Provided
1. PBS for sample dilution and washes
2. Container for PBS buffer
3. Micropipettes and disposable tips to apply patient samples
4. Humid chamber for incubation steps
5. Fluorescence microscope with 495nm exciter filter and 515nm barrier filter
6. Plastic squeeze bottle for initial wash in PBS
Additional components may be obtained from INOVA Diagnostics: PBS (508002), IFA System Negative Control (508186), FITC IgG (H&L), Monkey Adsorbed Conjugate (504011, 504071), 1% Evans Blue (504049) and PVA mounting medium (504046, 504047).

Test Procedure

Quality control

Positive and negative controls should be used every time samples are tested.

1. Mounting medium: Remove the mounting medium from the fridge to allow it to reach room temperature (18-28°C) before it is needed.
2. Dilute patient samples.
   - **Screening**: Dilute patient samples 1/5 by adding 40µL of serum to 160µL of PBS buffer.
   - **Titration**: Make serial dilutions of positive screened samples with PBS buffer (e.g. 1/10, 1/20, 1/40, 1/80 etc).
     For example: Take 100µL of the 1/5 dilution, mix with 100µL PBS to give a 1/10 dilution (repeat for further dilutions).
3. Substrate slides. Allow substrate slide(s) to reach room temperature (18-28°C) prior to removal from pouch(es).
   Label slides appropriately, place in the humid chamber and add positive and negative controls to appropriate wells. Add 50-100µL of diluted patient samples to the remaining wells.
4. Slide incubation. Incubate slides for 30 minutes in a humid chamber at room temperature (18-28°C).
5. PBS wash. Remove slides from humid chamber and rinse briefly with PBS squeeze bottle. Do not squirt directly on to the wells. Place slides in a rack and immerse in PBS and agitate or stir for 5-10 minutes.
6. Addition of fluorescent conjugate. Shake off excess PBS and blot around wells. Return slides to humid chamber and immediately cover each well with a drop of appropriately diluted fluorescent conjugate. DO NOT LEAVE WELLS UNCOVERED FOR LONGER THAN 15 SECONDS. Drying out of the substrate seriously affects the results. The use of a monkey adsorbed conjugate will greatly enhance results (e.g. 504011).
7. Slide incubation. Incubate slides for 30 minutes in humid chamber at room temperature (18-28°C), in the dark.
8. PBS wash. Wash again as described in step 5. OPTIONAL COUNTERSTAIN. Add 2-3 drops of 1% Evans Blue per 100mL of PBS prior to slide immersion.
9. Mounting with coverslip. Remove one slide at a time from PBS wash. Quickly dry around the wells and add a drop of mounting medium to each well. Carefully lower the slide onto the coverslip, avoiding air bubbles but, if present, do not attempt to remove. Wipe excess medium from around edge of coverslip.
10. View slides under fluorescence microscope. Slides may be stored for up to 3 days at 2-8°C, in the dark, without significant loss of fluorescence.

Results

Quality Control

A serum sample containing steroid cell autoantibodies should give bright apple-green fluorescent staining of the Leydig cells of the testis, the thecal cells of the ovary and the adrenal cortex. A negative control should show dull green staining in all tissues, with no discernible fluorescence. If controls do not appear as described, the test is invalid and should be repeated.

Interpretation of Results

See reference 5 for color photographic examples of these patterns. Results are reported as positive or negative. N.B: Each laboratory should establish at which point a positive result is considered clinically significant.

Limitations of the Procedure

1. The light source, filters and optics of different makes of fluorescence microscopes will influence the sensitivity of the kit. The performance of the microscope is significantly influenced by correct maintenance especially centering of the mercury vapor lamp and changing of the lamp after the recommended period of time.
2. The endothelial cells of capillaries in the testis and the ovary contain blood group substances and will be stained by anti-A and anti-B blood group antibodies. This staining could be confused with Leydig cell and theca cell staining respectively.
3. Suitability for use with other manufacturers' IFA reagents has not been assessed but use with such reagents should not necessarily be excluded.
4. This test alone should not be considered diagnostic. All other factors including the clinical history of the patients and other serological or biopsy results must also be taken into account.
Summary of the Procedure

1. Equilibrate mounting medium to room temperature.
2. Dilute PBS with distilled or deionized water.
3. Dilute patient sera 1/5 with PBS.
4. Equilibrate substrate slides to room temperature (18-28°C).
5. Apply 50-100 µL positive and negative controls and diluted patient sera to appropriate wells.
6. Incubate in a humid chamber for 30 minutes.
7. Wash for 5-10 minutes in PBS.
8. Blot around each well and immediately cover each well with a drop of conjugate.
10. Wash as step 7.
11. Mount.
12. View slide under fluorescence microscope.

References


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624220ENG December 2013
Revision 1