NOVA Lite® Cerebellum (Primate) Slides

For *In Vitro* Diagnostic Use

For Export Only. Not for sale in the United States.

Product Code: 508357, 508357.10

CLIA Complexity: High

Intended Use

Monkey cerebellum sections are intended for use in indirect immunofluorescence assays (IFA), screening human serum for circulating antibodies to Purkinje cells and other neurones of the cerebellum as an aid in the diagnosis of some paraneoplastic syndromes mainly arising from tumours of the lung, breast and ovary.

Summary and Explanation of the test

A small proportion of patients with paraneoplastic syndromes, particularly those associated with small cell lung carcinomas and ovarian/breast tumours, produce autoantibodies that react not only with their own tumour but also with neuronal tissues. Anti-cerebellar autoantibodies can readily be detected by indirect immunofluorescence on rodent, monkey or human cerebellum. There are two main groups of antibodies, those that stain the cytoplasm of Purkinje cells and those that stain the nuclei of Purkinje cells and other neurones. There is considerable variability in both the staining and the clinical features but the identification of high titre antibodies in patients with typical features is of value both in diagnosis and clinical management. Antibodies may occasionally be found in the absence of apparent tumours so the results must always be viewed in the context of the total clinical picture.

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-labelled 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.[1]

Reagents

Monkey cerebellum section on 4-well slides individually wrapped in a foil pouch containing desiccants

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[3], 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 titres or unclear staining patterns may occur.

Procedure

Materials provided

1. **508357** 1 x Cerebellum (Primate) Slide (4-well)

   Or

2. **508357.10** 10 x Cerebellum (Primate) Slide (4-well)
3. 1 - Instruction leaflet

Additional Materials Required But Not Provided

Distilled water to dilute PBS concentrate
Container for PBS buffer
Micropipettes and disposable tips to apply patient samples
Humid chamber for incubation steps
Fluorescence microscope with 495nm exciter filter and 515nm barrier filter
Plastic squeeze bottle for initial wash in PBS
Additional components may be obtained from INOVA Diagnostics: PBS (508002), IFA System Negative Control (508186), ANNA-1 Positive Control (504002), PCA-1 Positive Control (504509), FITC IgG (H&L) monkey adsorbed Conjugate (504011, 504071), 1% Evan's Blue (504049) and Mounting Medium (508001, 508005, 508006).

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 1/50 by adding 10µL of serum to 490µL PBS and a 1/500 by adding 5µL of serum to 2495µL PBS.
   Titration: Make serial dilutions of positive screened samples with PBS buffer (e.g. 1/50, 1/100, 1/200 and 1/400 etc).
   For example: Take 100µL of the 1/50 dilution, mix with 100µL PBS to give a 1/100 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, 504071).
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 Purkinje cell autoantibodies should give bright apple-green fluorescent staining of Purkinje cell cytoplasm.
A serum sample containing ANNA-1 autoantibodies should give apple-green granular fluorescent staining of most neuronal nuclei in the cerebellum, including Purkinje cells.
A negative control should show dull green staining in all the tissue, with no discernible fluorescence.
If controls do not appear as described, the test is invalid and should be repeated.

Interpretation of Results

See reference 2 for colour photographic examples of this pattern. Results are reported as positive or negative.
It is recommended when screening for paraneoplastic autoantibodies that patient samples which give a positive result at 1/50 should be repeated at 1/500. Only higher titres of greater than 1/100 are normally considered clinically significant on monkey cerebellum tissue.
N.B: Each laboratory should establish at which point a positive result is considered clinically significant.

References

1. Weller T. H. & Coons A. H. (1954). Fluorescent antibody studies with agent of Varicella and Herpes Zoster propagated in vitro: Proc. Soc. Exp. Biol. Med. **86**: 789-794.
2. Bradwell A. R. *et al* (2008). Atlas of Tissue Autoantibodies. Publ. The Binding Site Ltd., Birmingham UK.
3. Protein Reference Handbook of Autoimmunity (3rd Edition) 2004. Ed. A. Milford Ward, G.D. Wild. Publ. PRU Publications, Sheffield. 14.

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 centring of the mercury vapour lamp and changing of the lamp after the recommended period of time.
2. Suitability for use with other manufacturers' IFA reagents has not been assessed but use with such reagents should not necessarily be excluded.
3. This test alone should not be considered diagnostic. All other factors including the clinical history of the patient 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 water.
3. Dilute patient sera 1/50 and 1/500 with PBS.
4. Equilibrate substrate slides to room temperature (18-28°C).
5. Apply 50-100µL positive and negative controls and diluted patient sera to appropriate wells.
6. Incubate in a humid chamber for 20 minutes.
7. Wash for 5-10 minutes in PBS.
8. Blot around each well and immediately cover each well with a drop of conjugate.
9. Incubate as in step 6.
10. Wash as step 7.
11. Mount.
12. View slide under fluorescence microscope.

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