QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from non-specific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

RESULTS

A serum sample containing Purkinje cell auto-antibodies should give bright apple-green fluorescent staining of Purkinje cell cytoplasm.

A serum sample containing ANNA-1 auto-antibodies 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.

It is recommended when screening for paraneoplastic autoantibodies that patient samples which give a positive result at 1/50 should be repeated 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.

TEST LIMITATIONS

1. No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration. Among these host factors are age and sex. There is an increasing significance in positive ANA results in both males and females as age increases.
2. It is possible that substances such as drugs, viruses and bacteria may induce production of autoantibodies without there being any associated pathology or clinical condition.

LITERATURE 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. Protein Reference Handbook of Autoimmunity (3rd Edition) 2004. Ed. A Milford Ward, GD Wild. Publ. PRU Publications, Sheffield.

INSTRUCTIONS FOR USE

Anti-Neuronal Antibody Test System

K5029 – 48 tests

INTENDED USE

The Bio-Diagnostics anti-neuronal antibody test system is 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

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.

PRINCIPLE

Diluted human sera are incubated on the tissue sections. The primary test reaction involves circulating antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period while the serum covers the antigen surface.
A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence. With a positive reaction, the nuclear pattern appears apple-green when viewed under a fluorescent microscope, whilst a negative reaction appears black or greenish-black.

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WARNINGS AND PRECAUTIONS

1. Do not use components beyond their expiration date.
2. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
3. For in vitro diagnostic use.
4. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
5. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artefacts.

KIT CONTENTS

SLIDE	Monkey Cerebellum substrate slide (S5229)
CONJ **IgG**	FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502.This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL +	Hu (ANNA1) Positive Control no: C038N-0.5
CONTROL -	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA **PBS**	Buffer Pack no: R002
MM	Mounting Medium no: R005

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes and rack or microtitre system		Disposable pipettes
	Staining Dish and Slide Forceps	
Moisture Chamber	Volumetric Flask (500 ml)	Distilled Water
Fluorescence Microscope	Paper Towels – lint free	

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer	Contains sufficient for <n> tests	**RFU**	Ready for use
Temperature limitation	**IVD** In vitro diagnostic medical device		

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.
Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

Anti-Neuronal Antibody Test System

TEST INSTRUCTIONS

Screening: dilute test serums 1/50 (1 part patient sample to 49 parts diluent) and 1/500 (1 part patient sample to 499 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.
Titration: set up doubling dilutions of serum starting at 1/50, (i.e. 1/50, 1/100, 1/200, 1/400 etc.).

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (40-50µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 18-24ºC).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Remove slide from PBS and carefully wipe the underneath and around the wells with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. **Do not allow tissue to dry.**
7. Deliver 1 drop (40-50µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.
   Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.