NUFFIELD DEPARTMENT of MEDICINE

INFECTION, IMMUNITY IMMUNOPHENOTYPING

Anti Nuclear Antibody assay

The ANA assay is performed using a commercially available (Immuno Concepts, N.A. Ltd) fluorescent ANA test system, which has been adapted for high throughput screening of mouse serum.

The details of the Immuno Concepts assay can be found
http://www.menarinidiag.co.uk/Products/Autoimmunity/ZENIT-Reagents

8 (A-H) slides are used in one sample set with 9 samples being performed per 12 well slide making for a total of 72 samples per set.

Protocol for performing ANA

1. Dilute serum 1/5 in PBS (20µl PBS 5µl serum) and store at -20°C. Samples are diluted in sets of 9 on a 96 well plate, (e.g. A1-A9, B1-B9…)
2. Before adding to slides further dilute 1/20 in PBS, final dilution 1/100 (5 µl of 1:5 dilution and 95 µl of PBS).
3. Add 30µl of 1/100 dilution of serum to the Hep2 ANA slides straight out of packaging (no need to soak slides first). *NB: the slides have 12 wells, the first 9 wells have test serum added with wells 10-12 being used for control serum. For controls, use 30µl each of a positive control. We use a mouse with known strong ANA positivity, scored 3+, the Sanroque mouse* (Vinuesa et al. 2005) *a negative control serum from a C57Bl/6j mouse without positivity and a blank PBS well.*
4. Incubate 30 minutes, dark humidified container, RT
5. Wash slides first with squirt bottle of PBS, being careful not to squirt directly onto the wells. Then soak slides in PBS for 10mins, changing PBS once.
6. Remove slides one at a time and dip 3-5 times in distilled H2O and then tap slides on paper towel to dry (do not wipe around wells with towel)
7. Add 30ml Goat anti-mouse IgG (H + L) - FITC (1/500 dilution) to each well. Repeat with next slide. Antibody sourced from Invitrogen Cat. No. 626511from.
8. Incubate 20 minutes, dark humidified container, RT
9. Repeat wash steps 5 and 6
10. Add mounting fluid to slide, about 5 drops, then place cover slip on top
11. Store slide in slide book at 4°C. Slides can be kept up to 5 days before viewing with microscope.

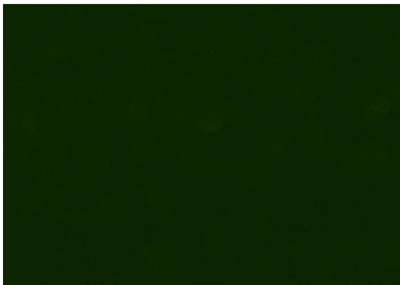
Reporting ANA intensity

The ANA Hep2 Slides are visualized on a Nikon wide-field TE2000U microscope. The microscope settings are fixed to minimize variation between sample batches.

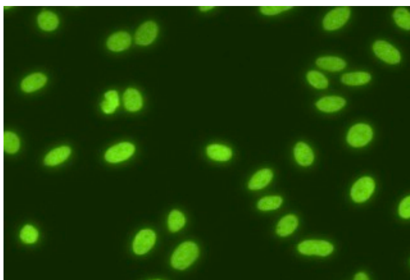
Microscope setting:
GFP
Magnification: 20x
Capture: 12 bit no binning
Contrast gain: 150
Auto exposure: 400ms

Results

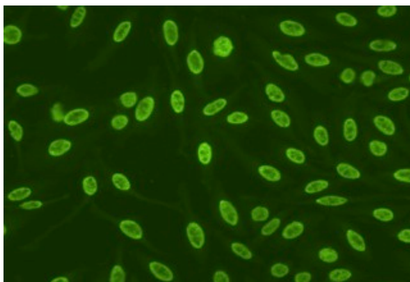
Negative: A serum is considered negative for antinuclear antibodies if nuclear staining is less than or equal to the negative control well with no clearly discernible pattern. The cytoplasm may demonstrate weak staining, with brighter staining of the non-chromosomal region of mitotic cells, but with no clearly discernible nuclear pattern.



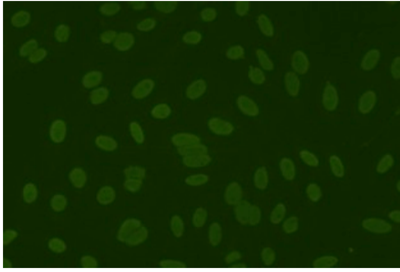
Positive: A serum is considered positive if the nucleus shows a clearly discernible pattern of staining in a majority of the interphase cells.



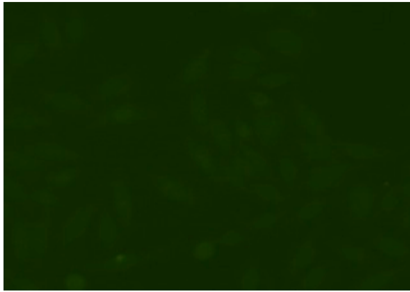
4+ Brilliant yellow-green (maximal fluorescence): clear-cut cell outline; sharply defined cell center.



3+ Less brilliant yellow-green fluorescence: clear-cut cell outline; sharply defined cell center.



2+ Definite cell pattern but dim fluorescence: cell outline less well defined.



1+ Very subdued fluorescence: cell outline almost indistinguishable from cell center in most instances. Demonstrates more fluorescents than negative control

A standard slide for the determination of these fluorescent intensities, FITC QC Slide TM, (Immuno Concepts, N.A. Ltd catalog number 1900) is used as standard for judging relative intensity.

0.5 has been used an indicator of a sample that did not make the 1+ (positive) criteria but still had some fluorescence.

Note: it is difficult to judge samples against each other from between sets. Differences in the microscope / bulb or the reagents used can result in higher or lower fluorescence. Therefore samples were judged against the control slide and slide specific sample controls on their respective day of assay.

Vinuesa, C.G. et al., 2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature*, 435(7041), pp.452–458.