From ANA to ENA: detection algorithms in the Netherlands

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European Autoantibody Standardization Initiative

Goals:
• Optimizing communication between clinicians and laboratory specialists,
• Establishing international standard preparations for autoantibody tests,
• Harmonizing testing algorithms.

Shoenfeld et al. ANYAS 1109: 138-144 (2007)
Damoiseaux et al. ANYAS 1173: 10-14 (2009)
Questionnaire

Categories:
• Organisation (n=4),
• ANA testing (n=14),
• Anti-dsDNA ab testing (n=8),
• Anti-ENA ab testing (n=15),
• ANA/ENA algorithm (n=16).
Response

Send out to 81 laboratories:
• Dutch diagnostic laboratories (n=76),
• Foreign diagnostic laboratories (n=2),
• Diagnostic compagnies (n=3),

→ 66 questionnaires were returned (87%)
Organisation

- UMC (7)
- General Hospital (50)
- Laboratory for GP's (3)
- Other (4)
- Combination (2)
Organisation

Organisation
- UMC (7)
- General Hospital (50)
- Laboratory for GP's (3)
- Other (4)
- Combination (2)

Laboratory type
- Immunology (10)
- Chemistry (40)
- Microbiology (7)
- Other (3)
- Combination (6)
Initiated and supported by

8

Organisation

- UMC (7)
- General Hospital (50)
- Laboratory for GP's (3)
- Other (4)
- Combination (2)

Laboratory type

- Immunology (10)
- Chemistry (40)
- Microbiology (7)
- Other (3)
- Combination (6)

Laboratory specialist

- Immunologist (20)
- Chemist (39)
- Microbiologist (5)
- Other (2)

Accreditation

- Yes (59)
- Ongoing (4)
- No (3)
ANA testing

- Yes (58)
- No (8)
ANA methods

ANA testing

- Yes (58) - 88%
- No (8) - 12%

ANA Methods

- IIF (43) - 74%
- FEIA (6) - 9%
- ELISA (5) - 7%
- Other (4) - 10%
ANA testing

ANA Methods

- IIF (43) 74%
- FEIA (6) 9%
- ELISA (5) 10%
- Other (4) 7%

ANA Screen titer

- 1/40 (17) 40%
- 1/80 (23)* 53%
- Other (3) 7%
ANA testing

ANA Methods
- IIF (43) 74%
- FEIA (6) 9%
- ELISA (5) 10%
- Other (4) 7%

ANA Screen titer
- 1/40 (17) 7%
- 1/80 (23)* 53%
- Other (3) 40%

ANA Reading
- 1 person (2) 5%
- 2 persons independent (39) 90%
- 2 persons in discussion (2) 5%
ANA titration

ANA Screen titer

- 1/40 (17) [40%]
- 1/80 (23)* [53%]
- Other (3) [7%]

ANA Titration

- Yes (3) [18%]
- No (14) [82%]
ANA titration

ANA Screen titer

- 1/40 (17)
- 1/80 (23)*
- Other (3)

ANA Titration

- Yes (3)
- No (14)

ANA Titration

- Yes (10)
- No (13)
ANA titration

ANA Screen titer

- 7% 1/40 (17)
- 40% 1/80 (23)*
- 53% Other (3)

ANA Titration

- 18% Yes (3)
- 82% No (14)

Distribution Titration Endpoint

- 43% Yes (10)
- 57% No (13)
ANA pattern

ANA Fluorescent pattern

- Yes (45)
- No (1)
ANA pattern

ANA Fluorescent pattern

- Yes (45)
- No (1)

98%

2%

ANA Pattern report

- Yes (24)
- Partial (13)*
- No (8)

53%

29%

18%
ANA pattern

ANA Fluorescent pattern

98%
Yes (45)
No (1)

ANA Pattern report

53% 29% 18%
Yes (24) Partial (13)* No (8)

ANA Pattern distinction

44 44 40 45 44 27 17
homogenous speckled SSA/atyp speckl centromere nucleolar cytoplasmic other
**ANA pattern**

**ANA Fluorescent pattern**
- Yes (45)
- No (1)
- 98%
- 2%

**ANA Pattern report**
- Yes (24)
- Partial (13)*
- No (8)
- 53%
- 29%
- 18%

**ANA Pattern distinction**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear dots</td>
<td>13</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>10</td>
</tr>
<tr>
<td>Nuclear matrix</td>
<td>4</td>
</tr>
<tr>
<td>PCNA</td>
<td>4</td>
</tr>
</tbody>
</table>
Anti-dsDNA ab testing

anti-dsDNA ab Testing

- Yes (63)
- No (3)
Anti-dsDNA ab screening

anti-dsDNA ab Testing

- Yes (63): 95%
- No (3): 5%

anti-dsDNA ab Screening

- Yes (13): 21%
- No (42): 66%
- Not reported (8)*: 13%
Anti-dsDNA ab screening

anti-dsDNA ab Testing
- Yes (63)
- No (3)

95%
5%

anti-dsDNA ab Screening
- Yes (13)
- No (42)
- Not reported (8)*

Method for screening
- Crithidia (4)
- ANA (7)
- Other (2)

54%
15%
31%
Anti-dsDNA ab methods

Method for detection

- Crithidia (10)
- Farr (2)
- FEIA (34)
- ELISA (5)
- Other (2)
- Combination (9)

anti-dsDNA ab Testing

- Yes (63)
- No (3)
Antidi-dsDNA ab methods

Method for detection

- Crithidia (10)
- Farr (2)
- FEIA (34)
- ELISA (5)
- Other (2)
- Combination (9)

<table>
<thead>
<tr>
<th>Method</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crithidia</td>
<td>7</td>
</tr>
<tr>
<td>Farr</td>
<td>6</td>
</tr>
<tr>
<td>FEIA</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
</tbody>
</table>
Anti-dsDNA ab report

anti-dsDNA ab Testing

- Yes (63)
- No (3)

anti-dsDNA ab Report

- Qualitative (16)
- Semi-quantitative (6)
- Quantitative (35)
- Combination (5)
- Not reported (1)
Anti-dsDNA ab report

anti-dsDNA ab Testing
- Yes (63)
- No (3)

anti-dsDNA ab Report
- Qualitative (16)
- Semi-quantitative (6)
- Quantitative (35)
- Combination (5)
- Not reported (1)

Units for anti-dsDNA ab (FEIA)

- IU/mL
- kU/L
- U/mL
- IU/L
- IE/mL
- Not reported
Relation between ANA and anti-dsDNA ab

- 7 labs consider ANA a screening assay for anti-dsDNA ab,
- 11 labs (17%) add ANA if anti-dsDNA ab is requested,
- 11 labs (17%) do not perform anti-dsDNA ab test if ANA is negative,
- 36 labs (55%) add anti-dsDNA ab if ANA is positive,
- 14 of these labs add anti-dsDNA ab only if ANA reveals a homogenous pattern,
- In 19 labs (29%) there seems to be no algorithm for ANA in relation to anti-dsDNA ab.
Anti-ENA ab testing

- Yes (62) 94%
- No (4) 6%
Anti-ENA ab screening

anti-ENA ab testing
- Yes (62) 94%
- No (4) 6%

anti-ENA ab screening
- Yes (54) 87%
- No (8) 13%
Anti-ENA ab screening

anti-ENA ab testing
- Yes (62)
- No (4)

94% 6%

Method for screening
- FEIA (40)**
- ELISA (7)*
- Alegria (2)
- Other (5)

74% 13% 4% 9%

anti-ENA ab screening
- Yes (54)
- No (8)
Anti-ENA ab methods

Method for detection

- ELISA (6)
- Line-blot (10)
- Dotblot (6)
- FEIA (32)
- Other (1)
- Combination (7)*

anti-ENA ab testing

- Yes (62) 94%
- No (4) 6%
Anti-ENA ab methods

Method for detection

- ELISA (6)
- Line-blot (10)
- Dotblot (6)
- FEIA (32)
- Other (1)
- Combination (7)*

ENA antigen detection

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Number of Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA</td>
<td>61</td>
</tr>
<tr>
<td>SSB</td>
<td>61</td>
</tr>
<tr>
<td>Sm</td>
<td>61</td>
</tr>
<tr>
<td>RNP</td>
<td>61</td>
</tr>
<tr>
<td>CENP-B</td>
<td>52</td>
</tr>
<tr>
<td>Scl-70</td>
<td>57</td>
</tr>
<tr>
<td>Jo-1</td>
<td>55</td>
</tr>
</tbody>
</table>
Anti-ENA ab methods

ENA report

- Qualitative (46)
- (Semi)quantitative (14)
- Not reported (2)

anti-ENA ab testing

- Yes (62) 94%
- No (4) 6%

Not reported (2)
Anti-ENA ab methods

- **ENA report**
  - Qualitative (46)
  - (Semi)quantitative (14)
  - Not reported (2)

- **anti-ENA ab testing**
  - Yes (62)
  - No (4)

- **ENA report**
  - All antigens (51)
  - Positive antigens only (8)*
  - Not reported (3)
Distinction SSA60 and Ro52

- Yes (17) - 28%
- No (44) - 72%
Distinction SSA60 and Ro52

- Yes (17)
- No (44)

Report of SSA60 and Ro52

- SSA60+ or Ro52+ = SSA (5)
- SSA60+ = SSA (1)
- SSA60+ = SSA60 / Ro52+ = Ro52 (8)
- Ro52 only if SSA60 is neg (2)
- Other (1)
Distinction SmB and SmD

- Yes (10)*
- No (51)
Initiated and supported by Distinction SmB and SmD

Distinction SmB and SmD

84% Yes (10) *
16% No (51)

Report of SmB and SmD

SmB+ or SmD+ = Sm (2)
SmD+ = Sm (7)
SmD+ = SmD (1)
Relation between ANA and anti-ENA ab

- 17 labs (26%) add ANA if anti-ENA ab is requested,
- 21 labs (32%) do not perform anti-ENA ab test if ANA is negative,
- 40 labs (61%) add anti-ENA ab if ANA is positive,
- 7 of these labs add anti-ENA ab only if ANA reveals a specific pattern and/or titer,
- In 14 labs (21%) there seems to be no algorithm for ANA in relation to anti-ENA ab.
Rapid testing

ANA Rapid test

- Yes (17)*
- No (34)
- Exceptional (6)
- Not reported (1)
Rapid testing

ANA Rapid test

- Yes (17)*
- No (34)
- Exceptional (6)
- Not reported (1)

anti-dsDNA ab Rapid test

- Yes (24)*
- No (35)
- Exceptional (3)
- Not reported (1)
Rapid testing

**ANA Rapid test**
- Yes (17)*
- No (34)
- Exceptional (6)
- Not reported (1)

**anti-dsDNA ab Rapid test**
- Yes (24)*
- No (35)
- Exceptional (3)
- Not reported (1)

**anti-ENA ab Rapid test**
- Yes (20)
- No (39)
- Exceptional (3)
Rapid testing

ANA Rapid test
- Yes (17)*
- No (34)
- Exceptional (6)
- Not reported (1)

anti-dsDNA ab Rapid test
- Yes (24)*
- No (35)
- Exceptional (3)
- Not reported (1)

anti-ENA ab Rapid test
- Yes (20)
- No (39)
- Exceptional (3)

Concordance Rapid testing
- Concordance Yes (20)
- Concordance No (41)
- No concordance (5)
### Time span for re-testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Diagnosis (n=13; 22%)</th>
<th>Follow-up (n=12; 20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANA</strong></td>
<td>n=8</td>
<td>n=8</td>
</tr>
<tr>
<td></td>
<td>2.1 months</td>
<td>3.4 months</td>
</tr>
<tr>
<td><strong>Anti-dsDNA ab</strong></td>
<td>n=5</td>
<td>n=4</td>
</tr>
<tr>
<td></td>
<td>1.7 months</td>
<td>1.6 months</td>
</tr>
<tr>
<td><strong>Anti-ENA ab</strong></td>
<td>n=12</td>
<td>n=10</td>
</tr>
<tr>
<td></td>
<td>5.8 months</td>
<td>5.8 months</td>
</tr>
</tbody>
</table>

*n=59*
Major findings

1. ~20% use ELISA/FEIA for ANA testing (n=11),
2. ~40% (IIF) use a start dilution of 1:40 and ~80% of these laboratories do not titrate ANA (n=14),
3. Almost all laboratories (98%) score the ANA pattern; only half of these (53%) report the pattern,
4. ~25% report anti-dsDNA ab only qualitatively (n=16),
5. The relation between a homogenous ANA pattern and anti-dsDNA ab is only poorly translated into a diagnostic algorithm (n=14),
6. Only 7 laboratories (11%) use different techniques for anti-ENA ab testing,

7. The majority (74%) reports anti-ENA ab in a qualitative way (n=46),

8. Eight labs (13%) only report positive results for anti-ENA ab,

9. The relation between ANA patterns and/or titers is only poorly translated into a diagnostic algorithm (n=7),
Major findings

10. Many laboratories are not aware of the antigen (SmD) composition of their ENA test system,
11. Allowing rapid testing for non-specific tests, like ANA (n=23) and anti-ENA ab (n=23), is disputable,
12. Allowing rapid testing for anti-dsDNA ab (n=26) should be extended,
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