AUTOIMMUNITY DIAGNOSTIC STRATEGIES
(ANA, anti-dsDNA, anti-ENA) IN ITALIAN CLINICAL LABORATORIES

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INTRODUCTION: ANA and related antibodies are fundamental for the diagnosis of autoimmune diseases. Since the request for autoantibodies is increased, new techniques, as well as testing-strategies, have been recently developed.

AIMS OF THE STUDY: The survey is a project of the "*European Autoimmunity Standardisation Initiative*" (E.A.S.I., www.easi-network.com), with the financial support of Phadia S.r.l., part of Thermo Fisher Scientific, aimed to investigate the daily practice in the different European countries, to find appropriate tools for managing systemic autoimmune rheumatic diseases (SARD).

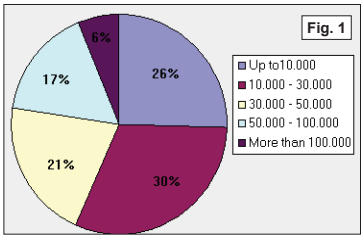
METHODS:

- a 68 multi-choice questions questionnaire was sent to more than 600 laboratory specialists, representative of 444 labs;
- the questionnaire was introduced by an e-mail, with the link to a web-site for the drawing up;
- returned polls from Labs performing Autoimmune Diagnostic Testing were identified and statistically evaluated by using the online software Survey Monkey (www.surveymonkey.com).

PRELIMINARY RESULTS

30% of the questionnaires was completed: most (74%) were from public and the remaining from private or academic Institutions, 60% of the laboratories were of large dimension (autoimmune tests performed yearly shown in Fig.1). Autoimmunity was only rarely independent, more often it was part of one of the major specialties (Clin. Biochemistry 44%, Central Lab 19%, Clin. Immunology 13%, Microbiology 10%). 70% of the laboratories taking part to the survey were quality certified. Medical doctors (50%) or biologists (46%) were in charge for the autoimmune diagnostics.

Fig. 1



ANA-testing: ANA were detected by IIF on HEp-2/HEp-2000 in more than 90% of the centers, sometimes followed by a 2nd assay (screening dilutions in Fig. 2).
IIF evaluation was done by two or more specialists in up to 60% of the labs.
Interestingly, in only two centers a digital image analysis system, followed by specialist control, was used. 91% of the +ve samples were diluted until 1:640 (36%), 1:1280 (31%), 1:2560 or more (33%). Recognized fluoroscopic patterns are listed in Fig. 3.
Titers & patterns were almost always reported to the clinicians.
Samples with exclusively cytoplasmic reaction were generally referred as "ANA negative, presence of cytoplasmic staining pattern".

Fig.2 ANA-IIF screening dilution

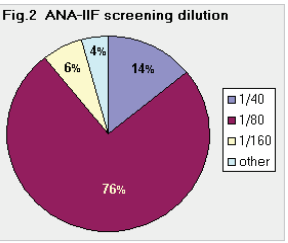
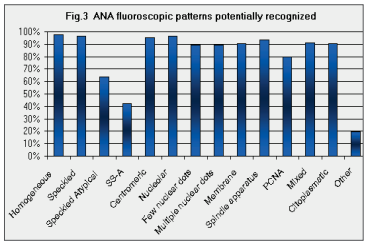


Fig.3 ANA fluoroscopic patterns potentially recognized



anti-dsDNA were detected with the most "appropriate" assay, and more than one method was used in two-third of the labs (Fig. 4a, 4b). Discordant results were reported as described in Fig. 5.

anti-dsDNA results were reported	Fig. 4b
qualitative (present / absent)	8,1%
semi-quantitative (titer)	35,5%
quantitative (UI/ml)	46,0%
quantitative (U/ml)	10,5%

Fig. 4a

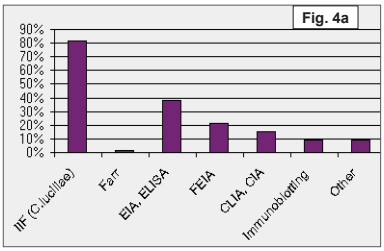
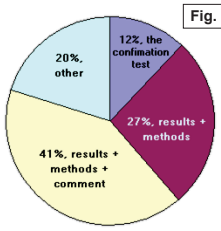
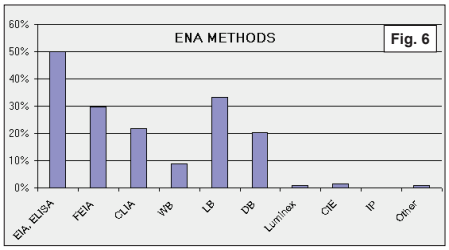


Fig. 5



ENA: were largely detected by a screening test (90%) using the methods in Fig. 6. Because a "gold standard" for all analytes is not available, 53% of the labs performed more than one assay/sample. Detected specificities listed in Fig. 7.

Fig. 6



ENA specificities routinarily detected	Fig. 7
SS-A/Ro (SS-A60 kDa e/o SS-A52 kDa)	98,4%
SS-B/La	96,8%
Sm/RNP	77,4%
Sm (SmB and/or SmD)	72,6%
RNP (70 kDa, A/C)	79,0%
Cenp-B	71,8%
Scl-70	95,2%
Jo-1	95,2%
Rib-P	37,1%
Other	17,7%

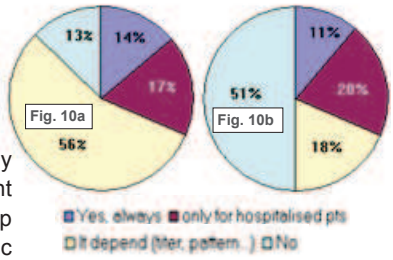
Testing strategy & Organization: although the regulatory constraints and with respect to other professionals, Italian labs showed a surprising autonomy in making decisions about which tests to perform in specific situations (Fig. 8-9). In-depht Analysis were added or removed to ANA, in order to pursue the "appropriateness" (Fig.10a, b).

testing options provided in the order form	Fig. 8
ANA	89,1%
ANA profile (ENA screen on +ve)	4,2%
ANA profile (ENA single specificities on +ve)	8,4%
ENA screen (ENA single specificities on +ve)	68,1%
ENA single specificities	40,3%
Anti-dsDNA	83,2%
Other	14,3%

Fig. 9

Are specific diagnostic algorithms applied for ANA/ENA testing?			
ANA	%	ENA	%
No	67%	No	66%
Yes, ANA automatically added to samples with ENA	13%	Yes, on ANA+ve samples	10%
Yes, ANA automatically added to samples with anti-dsDNA	3%	Yes, depending on ANA	14%
Other	17%	Other	10%

Addition of tests — Removal of tests



Rules defining the shortest time interval between 2 consecutive determinations of the same analyte were generally not applied. Clinical information were considered extremely useful, and strongly influenced the choice of different algorithms/test. When appropriate, more than 90% of the laboratory specialists added comments to help interpretation. Automation is largely diffused in Italian labs. Indeed, 90% of the IIFT and 99% of the immunometric assays were performed by slide-processors and/or automated systems.

CONCLUSIONS

Great changes have taken place in the organization of Italian medical labs in the last decade. Several tests, historically performed in specialized settings only, and later diffusely available, have been recently consolidated and centralized in large reference labs with areas devoted to sub-disciplines like autoimmunity. Noteworthy, most of the Italian labs performed autoimmunity-testing according to the International recommendations. They paid great attention to improve the cooperation with the clinicians, whose information on requests were strongly appreciated and considered. Finally, laboratory specialists showed a high consciousness of their professional expertise and commitments, by adding interpretative comments to clinical reports when useful.