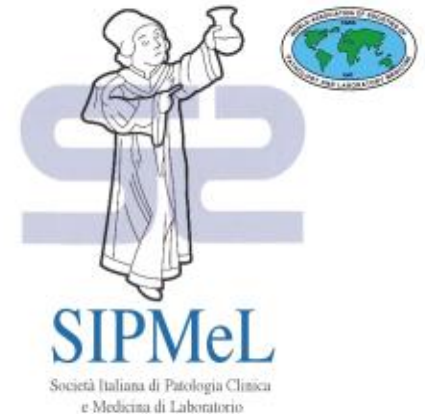
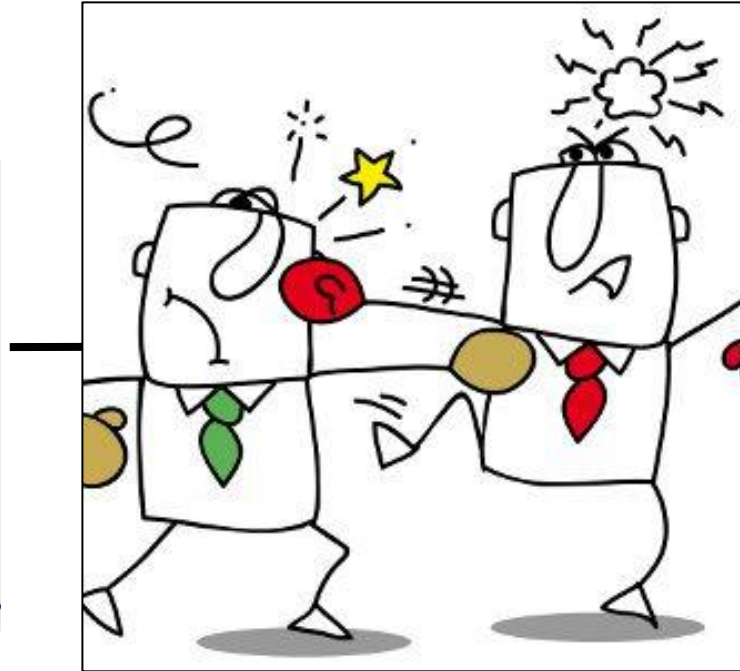


12th International Autoimmunity Seminar

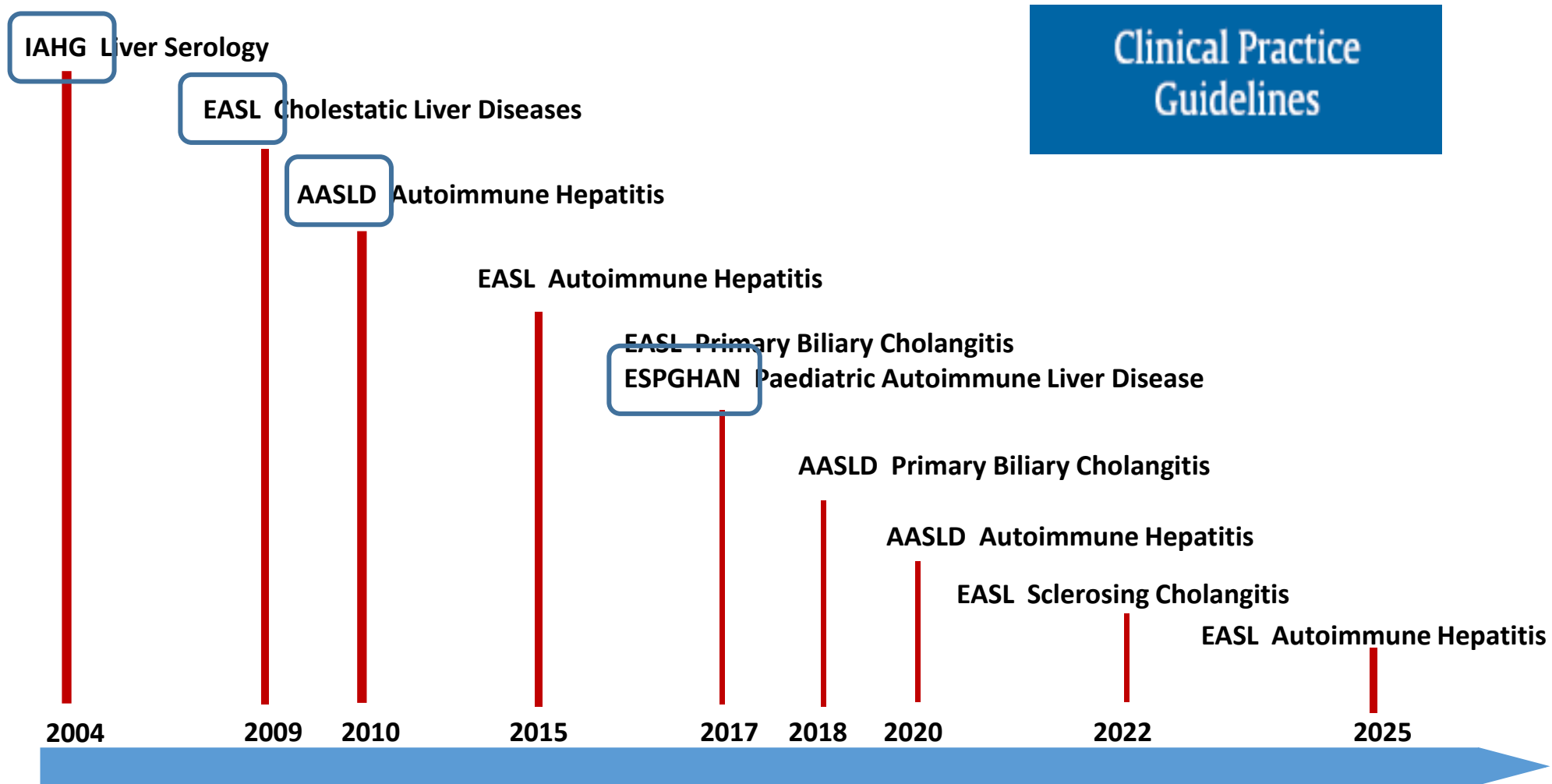
October 14-15, 2025 • Vilnius



Italian guidelines for the immunological diagnosis of autoimmune liver diseases: a step forward from EASL guidelines

Nicola Bizzaro

International guidelines from 2004



EASL = *European Association for the Study of the Liver*

AASLD = *American Association for the Study of Liver Diseases*

ESPGHAN = *European Society for Paediatric Gastroenterology Hepatology and Nutrition*

IAHG = *International Autoimmune Hepatitis Group*

Foreword

International guidelines, including those of the European Association for the Study of Liver Diseases (EASL), are of **extremely high quality** and **provide excellent recommendations** for the clinical diagnosis and treatment of autoimmune hepatitis and primary biliary cholangitis.

However, very often the items regarding serum immunological tests are not sufficiently updated and do not take into account the technological evolution of test methods.

Critical points

1. Analytical methods:

- Many autoantibodies are implicated in the diagnosis of AIH and PBC.
- Various analytical platforms are currently available for their detection.

2. Initial serum dilution (cutoff) for IIF-based methods:

- Depending on the type of antibody to be detected and the substrate used.
- Should it be differentiated in adults and children?

**Which method to
detect ANA?**

**Liver autoimmune serology: a consensus statement
from the committee for autoimmune serology of the International
Autoimmune Hepatitis Group[☆]**

Journal of Hepatology 41 (2004) 677–683

IAHG 2004

“**The basic technique for routine testing** of autoantibodies relevant to AIH, including ANA, **is IFA** on a **freshly removed (unfixed) rodent** multi-organ substrate panel (including kidney, liver and stomach)”

The use of **rodent substrate for screening ANA** for AIH was further emphasized in 2015 by the EASL practice guidelines on autoimmune hepatitis



EASL Clinical Practice Guidelines: Autoimmune hepatitis[☆]

European Association for the Study of the Liver^{*}

EASL 2015

Summary of current consensus/position statements (or criteria) for AIH

- ☐ In case of suspicion of autoimmune hepatitis, **ANA should be detected by IFA on rodent tissue** (liver, kidney, stomach).
- ☐ In case of positive ANA, the pattern should be defined by IFA on HEp-2 cells.

2010

Diagnosis and Management of Autoimmune Hepatitis

Michael P. Manns,¹ Albert J. Czaja,² James D. Gorham,³ Edward L. Krawitt,⁴ Giordina Mieli-Vergani,⁵
Diego Vergani,⁶ and John M. Vierling⁷

2020

Diagnosis and Management of Autoimmune Hepatitis in Adults and Children: 2019 Practice Guidance and Guidelines From the American Association for the Study of Liver Diseases

Cara L. Mack,¹ David Adams,² David N. Assis,³ Nanda Kerkar,⁴ Michael P. Manns,⁵ Marilyn J. Mayo,⁶ John M. Vierling,⁷
Mouaz Alsawas,⁸ Mohammad H. Murad,⁹ and Albert J. Czaja¹⁰

American Association for the Study of Liver Diseases

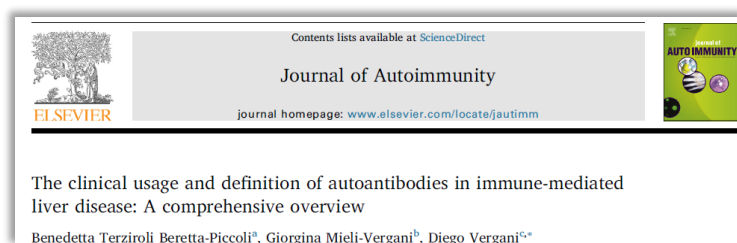
“The mainstay technique for autoantibody screening is indirect immunofluorescence on composite **sections of freshly frozen rodent stomach, kidney and liver**”.

..... and in 2018 by the **ESPGHAN**
Hepatology Committee

Diagnosis and Management of Pediatric Autoimmune
Liver Disease: ESPGHAN Hepatology Committee
Position Statement

2018

The ESPGHAN position statement did **not recommend the use of HEp-2 cells** to screen for ANA in AIH because of the relatively high positivity rate in the normal population and in infections, especially in children.



2018

Indirect immunofluorescence on triple rodent tissue remains the gold standard methodology to detect liver-relevant autoantibodies.

The use of HEp2 cells as a substrate for screening is not recommended, since by this methodology low titer (< 1:40) ANA is frequently detected in healthy individuals.

Automated assays require further studies before they can be used as a reliable alternative to immunofluorescence; currently, they may be used as complementary to immunofluorescence.

Indirect immunofluorescence is an old technique requiring expertise and patience that may not be ideal for the modern laboratory.
Yet, for the moment, it is here to stay.



Update of the simplified criteria for autoimmune hepatitis: Evaluation of the methodology for immunoserological testing

Johanna Galaski^{1,†}, Christina Weiler-Normann^{1,2,7,†}, Miriam Schakat¹, Kalliopi Zachou³,
Paolo Muratori^{4,7}, Sibylle Lampalzer¹, Friedrich Haag⁵, Christoph Schramm^{1,2,7}, Marco Lenzi^{6,7},
George N. Dalekos³, Ansgar W. Lohse^{1,7,*}

2021

“IFT on HEp-2 cells is a valid alternative to the standard ANA assessment on rodent tissue sections in AIH when cutoffs titers are increased”.

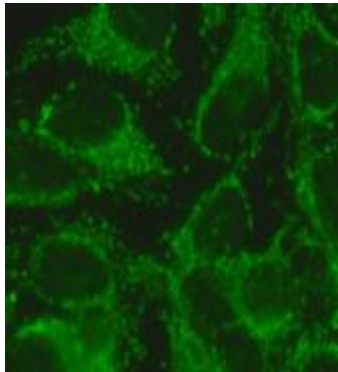
ANA IFT on liver sections: 83.6% sensitivity and 69.4% specificity at a titer of **1:40**.

ANA IFT on HEp-2 cells, 75.4% sensitivity and 73.6%, specificity at a titer of **1:160**.

The SIPMeL position 2023

We recommend the use of the IIF method on HEp-2 cells to search for ANA

1. HEp-2 cells are the substrate used in all laboratories worldwide for the ANA test.
2. Fluorescence patterns assessed on HEp-2 cells may be useful to guide further confirmatory tests aimed at identifying other ANA specificities such as sp100 and gp210, and even anti-mitochondrial antibodies (AMA), a serological hallmark of PBC.



The AMA-like pattern is often **detected incidentally** while performing ANA testing on HEp-2 cells (*case finding*).

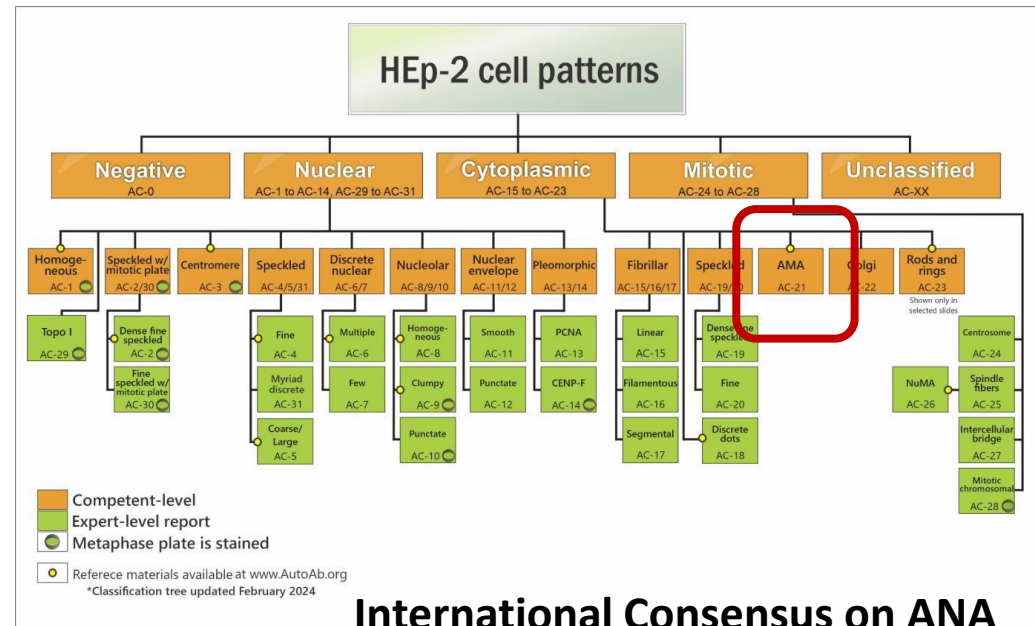
302 AMA + new cases: 39 (13%) on triple tissue and 263 (87%) on HEp-2.

Porcelli B, et al. Incidental finding of anti-mitochondrial antibody: A neglected entity needing reappraisal. Dig Liver Dis 2023:S1590-8658(23)00567-4.

Reporting cytoplasmic patterns as ANA positive or ANA negative

Laboratories using HEp-2 cells as substrate, may report cytoplasmic staining as either as ANApos or ANAneg. **Reporting a positive cytoplasmic pattern as ANA positive may lead to an incorrect score within the IAIHG diagnostic systems** (Terziroli, Vergani 2022).

Parameters/Features	Score
Female sex	+ 2
ALP:AST (or ALT) ratio:	
<1.5	+ 2
1.5–3.0	0
> 3.0	–2
Serum globulins or IgG above normal	
>2.0	+3
1.5–2.0	+2
1.0–1.5	+1
<1.0	0
ANA, SMA or LKM-1	
>1:80	+3
1:80	+2
1:40	+1
<1:40	0
AMA positive	–4



International Consensus on ANA Patterns

GdS-AI SIPMeL position

In case of a fluorescence pattern suggestive of AMA (either on triple rat tissue or on HEp-2 cells), it is necessary to perform a confirmatory test for the M2 antigen with higher specific methods such as ELISA, CLIA, FEIA or immunoblot.

International AIH Group
(IAIHG) diagnostic criteria for
autoimmune hepatitis

In patients with suspected autoimmune hepatitis, ANA screening tests can be performed using immunometric methods (so called CTD screen assays)?

Systematic reviews and meta-analyses have shown that new recently developed **solid-phase assays** for the screening of ANA are able to provide overall diagnostic performance that **are equivalent, or even better**, than those provided by indirect immunofluorescence on HEp-2 cells.

However, **the IIF method should still be used until solid-phase methods will be available to detect a greater number of autoantibodies including those targeting antigens that are important in the diagnosis of autoimmune liver diseases (sp100, gp210 and M2).**

Cinquanta L, et al. **Updating the Italian Society of Clinical Pathology and Laboratory Medicine guidelines on the use of autoantibody tests in the diagnosis and monitoring of systemic rheumatic autoimmune diseases.** Riv It Med lab 2019

SIPMeL recommendation 1.1



To date, immunometric methods to search for ANA are less sensitive than IIF on HEp-2 and therefore it is not recommended to use them in the screening phase of AIH.

**Which screening
titer for ANA?**

Special article

**Liver autoimmune serology: a consensus statement
from the committee for autoimmune serology of the International
Autoimmune Hepatitis Group[☆]**
Journal of Hepatology 41 (2004) 677–683

EASL Clinical Practice Guidelines: Autoimmune hepatitis[☆]

European Association for the Study of the Liver*



***IAHG 2004
AASLD 2010
EASL 2015***

**Diagnosis and Management of Pediatric Autoimmune
Liver Disease: ESPGHAN Hepatology Committee
Position Statement**

ESPGHAN 2018

The threshold for positive ANA should be 1:40 for adults and 1:20 for children on rodent tissue.

Screening dilution for ANA

On the HEp-2 cell line, these low dilutions (1:20 - 1:40) cannot be applied in clinical laboratories as they would greatly increase the possibility of false positive results in healthy individuals and in patients affected by non-autoimmune diseases.

(Sorrentino MC, et al. Riv It Med Lab 2023)

Update of the simplified criteria for autoimmune hepatitis: Evaluation of the methodology for immunoserological testing

Johanna Galaski^{1,†}, Christina Weiler-Normann^{1,2,7,†}, Miriam Schakat¹, Kalliopi Zachou³,
Paolo Muratori^{4,7}, Sibylle Lampalzer¹, Friedrich Haag⁵, Christoph Schramm^{1,2,7}, Marco Lenzi^{6,7},
George N. Dalekos³, Ansgar W. Lohse^{1,7,*}

2021

In the screening of AIH, at a cutoff of 1:160 ANA on HEp-2 cells show a sensitivity and specificity comparable to the dilution of 1:40 on triple rat tissue.

SIPMeL recommendation 1.2

In detecting ANA in IIF on HEp-2 cells, it is recommended to **use the initial dilution of 1:80.**

For the pediatric population, pending further studies on larger case series, it is recommended to use the same screening dilution proposed for the adult population and to apply the same evaluation criteria.

Overall quality of evidence: moderate
Strength of recommendation: strong

**Which method
to detect ASMA
and anti-LKM?**

In patients with suspected autoimmune hepatitis, the search for ASMA and anti-LKM antibodies can be performed with immunometric methods?

NO

F-actin specificity testing with ELISA methods showed negative results in 20% of sera in IIF.

Cause:

- ✓ reactivity directed against still unknown autoantigens?
- ✓ antigenic epitope modifications during protein purification or coating on the solid phase support?

Terziroli Beretta-Piccoli B, et al. 2018

YES

ELISA based anti-F-actin performed better than SMA assessment by IFT.

Conclusion: **ELISA-based** autoantibody evaluation for SMA is potential alternative to tissue-based IFT for the diagnosis of AIH.

Galaski J, et al. 2021

The SIPMeL position

The IIF method on triple rodent tissue sections remains the method of choice for screening ASMA and anti-LKM.

Overall quality of evidence: moderate

Strength of recommendation: strong

**Which method
to detect AMA?**

Current recommendations for AMA

EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis[☆]

European Association for the Study of the Liver*

2019

Diagnosis and Management of Primary Biliary Cholangitis

Zobair M. Younossi, MD, MPH, FACP, AGAF, FAASLD¹, David Bernstein, MD, FAASLD, FACP, AGAF, FACP², Mitchell L. Shiffman, MD³, Paul Kwo, MD⁴, W. Ray Kim, MD⁵, Kris V. Kowdley, MD⁶ and Ira M. Jacobson, MD⁷

2017

2018

Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases



Keith D. Lindor,¹ Christopher L. Bowlus,² James Boyer,³ Cynthia Levy,⁴ and Marilyn Mayo⁵

- Anti-mitochondrial antibodies should be screened for **by IFA on triple-substrate**. Anti-PDH-E2 antibodies can be confirmed by antigen-specific assays.

May HEp-2 cells be used to screen for AMA?

May immunometric methods be used to screen for AMA?

Clin Chem Lab Med 2020; 58(8): e142–e143

DE GRUYTER

Letter to the Editor

Brunetta Porcelli*, Lucia Terzuoli, Maria Romana Bacarelli, Francesca Cinci
and Nicola Bizzaro

How reliable is the detection of anti-mitochondrial antibodies on murine triple-tissue?

62 samples with an AMA-like (AC-21) pattern on HEp-2

	AMA on LKS positive	AMA on LKS negative
M2 positive (FEIA)	31 (50%)	11 (18%)
M2 negative (FEIA)	3 (5%)	17 (27%)

An AMA-like pattern on HEp-2 was not confirmed in 27% of cases
AMA-IIF on rodent tissues missed 18% of true positives

SIPMeL recommendation 2.1

RACCOMANDAZIONI E LINEE GUIDA

Linee guida SIPMeL per la determinazione
degli autoanticorpi nella diagnosi
delle malattie autoimmuni del fegato

Italian Society of Clinical Pathology and Laboratory
Medicine guidelines on the use of autoantibody tests
in the diagnosis of liver autoimmune diseases

The IIF method on triple tissue sections still represents a reliable and convenient methodological approach for the search for AMA.

However, if available in the laboratory, the search for AMA can be performed directly with ELISA, CLIA, FEIA or immunoblot, because these methods guarantee greater diagnostic specificity with the same sensitivity.

Overall quality of evidence: high

Strength of recommendation: strong

**Which screening
titer for AMA,
ASMA and LKM?**

Screening dilution for tests on LKS

Diagnostic criteria of autoimmune hepatitis



Rodrigo Liberal ^{a,b}, Charlotte R. Grant ^a, Maria Serena Longhi ^a, Giorgina Mieli-Vergani ^a, Diego Vergani ^{a,*}

2014

^a Paediatric Liver, GI & Nutrition Centre and Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, London, UK

^b Faculty of Medicine, University of Porto, Porto, Portugal

Autoimmunity Reviews 13 (2014) 435–440

- **1:40 for ASMA and for anti-LKM in adults**
- **1:10 for ASMA and for anti-LKM in children**

Clinical Practice Guidelines



2015

EASL Clinical Practice Guidelines: Autoimmune hepatitis[☆]

European Association for the Study of the Liver^{*}

- **1:40 for ASMA and for anti-LKM in adults**
- **1:20 for ASMA and 1:10 for anti-LKM in children**

Adults

ASMA 1:40

LKM 1:40

Children

ASMA 1:20

LKM 1:10

Critical aspects in choosing the screening dilution for ASMA

Current LED microscopes that have replaced those with mercury lamps are equipped with **high sensitivity** (**greater brightness** of the preparations) and increased image sharpness, also thanks to the use of new conjugates.

Autoantibody Profiling in a Cohort of Pediatric and Adult Patients With Autoimmune Hepatitis

ASMA on triple tissue, at the **1:40 dilution**, presented the same diagnostic sensitivity in both adults and children.

Villalta D, et al. 2014

Update of the simplified criteria for autoimmune hepatitis:
Evaluation of the methodology for immunoserological testing

At a dilution of 1:40:

All ASMA patterns (V and/or G and/or T)

Sensitivity 86.9% - specificity 37.5%

At a titer of 1:80:

Sensitivity 80.3% - specificity 69.4%

Galansky J, et al. 2021

SIPMeL recommendation 1.5

RACCOMANDAZIONI E LINEE GUIDA

Linee guida SIPMeL per la determinazione
degli autoanticorpi nella diagnosi
delle malattie autoimmuni del fegato

Italian Society of Clinical Pathology and Laboratory
Medicine guidelines on the use of autoantibody tests
in the diagnosis of liver autoimmune diseases

In the case of suspected autoimmune hepatitis, for the detection of ASMA in IIF on triple murine tissue sections, a screening dilution of **1:40** is suggested in laboratories where subjects with **a high pre-test probability** of AIH are present, and **1:80 in laboratories where the pre-test probability is much lower**.

For the **pediatric population**, pending further studies on larger case series, it is recommended to use **the same screening dilution** proposed for the **adult population** and to apply the same evaluation criteria previously exposed.

Overall quality of evidence: moderate

Strength of recommendation: weak

***Study Group on Autoimmunology of the Italian Society of
Clinical Pathology and Laboratory Medicine***

