

URUGUAY: EXTERNAL QUALITY ASSESSMENT SURVEY FOR THE STANDARDIZATION OF ANTINUCLEAR-CYTOPLASMICS ANTIBODIES

PACULTAD DE OUIMICA

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INTRODUCCION

Uruguay is a small country with no more than 160 laboratories, without a consensus for de interpretation of Antinuclear-Cytoplasmic Antibodies (ANA). Among those, not more than thirty perform the determination of ANA by indirect immunofluorescence (IFI). In November/2012 we organized a meeting for reading and interpretations of ANA; a activity that were proposed was to assess and report images of different patterns. The result of this activity was the need for an External Quality Assessment Survey (EQAS) for ANA.

OBJETIVE

The aim of this work is to present the development of an EQAS for ANA in Uruguay, without a consensus for ANA, and present the first results.

METHODOLOGY

Sera from patients with positive ANA were sent to each laboratory. Three samples sent in 2013 (September/October/November), two in 2014 (April/July). In this work we present the results from two samples (Set/2013 and April/2014). Each laboratory should perform analysis by indirect immunofluorescence as a routine sample. The report was send via e-mail in spreadsheet Excel created for this purpose. Each report must include the laboratory code, sample code, commercial kits, and the following data (with restricted option for response):

Methodic data	option for response
	Mercury 20W, Mercury 50W,
Type of lamp	Mercury 100w, Halogen, LED
Conjugate type	IgG, IgG-IgA-IgM
Type of sample processing	Manual, Automated
Initial dilution	1/40, 1/80, 1/160

Sample report	option for response	
Result	Negative, Positive	
Title	1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560, 1/3280, 1/5120	

Fluorescence detected	option for response
Nuclear	Yes, No
Nucleolar	Yes, No
Cytoplasmatic	Yes, No
Chromatids	Yes, No
Mitotic Spindle Apparatus	Yes, No
Nuclear Membrane	Yes, No

Nuclear fluorescence type	option for response
Homogeneous	Yes, No
Speckled	Yes, No
Multiple nuclear dots	Yes, No
Different between cells (Pleomorphic)	Yes, No

Cytoplasmic fluorescence type	option for response
Isolate dots	Yes, No
Reticular	Yes, No
Diffuse	Yes, No
Lines	Yes, No
Small segment	Yes, No

Chromatids fluorescence type	option for response
Homogeneous	Yes, No
Speckled	Yes, No

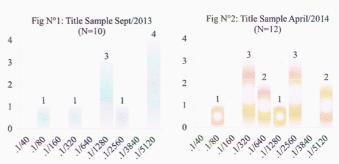
Mitotic Apparatus fluorescence in	option for response
Centriole	Yes, No
Mitotic Spindle	Yes, No
Intercelular bridge	Yes, No

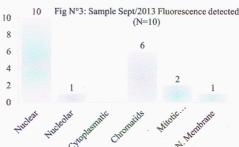
In this site write the final reports for this sample

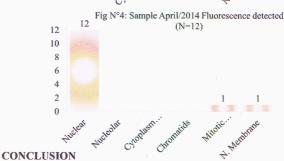
RESULTS

-The participant laboratory used five different type of commercial kits, the majority of them with IgG conjugate type. None of the laboratories use microscope with mercury 20w lamp. The initial dilution used is 1/40 or 1/80. -All of the participant (N=10) reports positive the sample send on Sept/2013 and classified the nuclear fluorescence as homogeneous. Title profile show in Fig N°1 and fluorescence type reports in the different cell structure in Fig N°3.

-Twelve of the thirteen participant reports positive the sample send on April/2014 and classified the nuclear fluorescence as speckled. Title profile show in Fig $N^{\circ}2$ and fluorescence type reports in the different cell structure in Fig $N^{\circ}4$.







-In few months we had good adhesion to EQAS program for ANA, and we have already enlisted in 2014 most of the laboratories.

-Evaluating the reports of the sample send in Sept/2013 (Nuclear Homogeneous with anti-dsDNA positive (ELISA)) found: all participants reported positive, nuclear fluorescence positive, and classified as homogeneous. However some participating also participants who reported fluorescence in nucleoli, mitotic apparataus and nuclear membrane. In the total of the participant: eight reports titles between 1/1280-1/5120, one 1/320, and one laboratory did not perform the titration of the sample (1/80)

-Evaluating the reports of the sample send in April/2014 (Nuclear Speckled) found: twelve of the thirteen participants reported positive, nuclear fluorescence positive, and classified as speckled. In the total of the participant: eleven reports titles between 1/320-1/5120, and one laboratory did not perform the titration of the sample (1/80).

-When evaluated the final reports of both samples from each laboratory, we can detect the use of different expression to explain the same fluorescence image. (Data not show)