



INTI

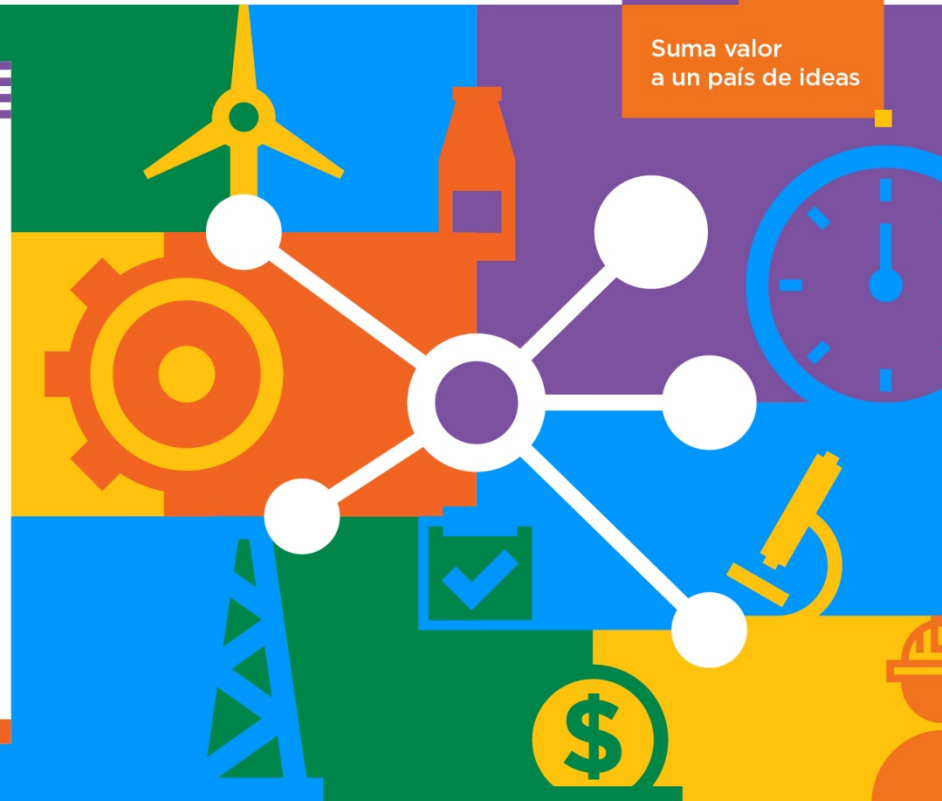
Instituto
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Ministerio de Producción y Trabajo
Presidencia de la Nación

DEVELOPMENT OF A PRODUCTION PROCESS FOR A CANDIDATE BSA REFERENCE MATERIAL.

Suma valor
a un país de ideas



THIS IS A REGIONAL PROJECT



•INMETRO, Brazil, 27-29 June 2017.



OBJECTIVE

Develop a highly-pure traceable BSA preparation and Certify it, according to ISO norms 17.034.

USES

Standardization of Clinical analysis for “quantification of total serum proteins” or total protein content for Biotechnological productions, mainly applied to colorimetric methods.



DEVELOPMENT OF A PRODUCTION PROCESS FOR A CANDIDATE BSA REFERENCE

MATERIAL

Four different processes and products were tested during the development of Candidate RM.

R.M. 1

R.M. 2

R.M. 3



Purity around 95%

Purity \geq 98,5%

PRE-PURIFIED BSA SERUMS. FRACTION V

OR SIMILAR FRACTIONS

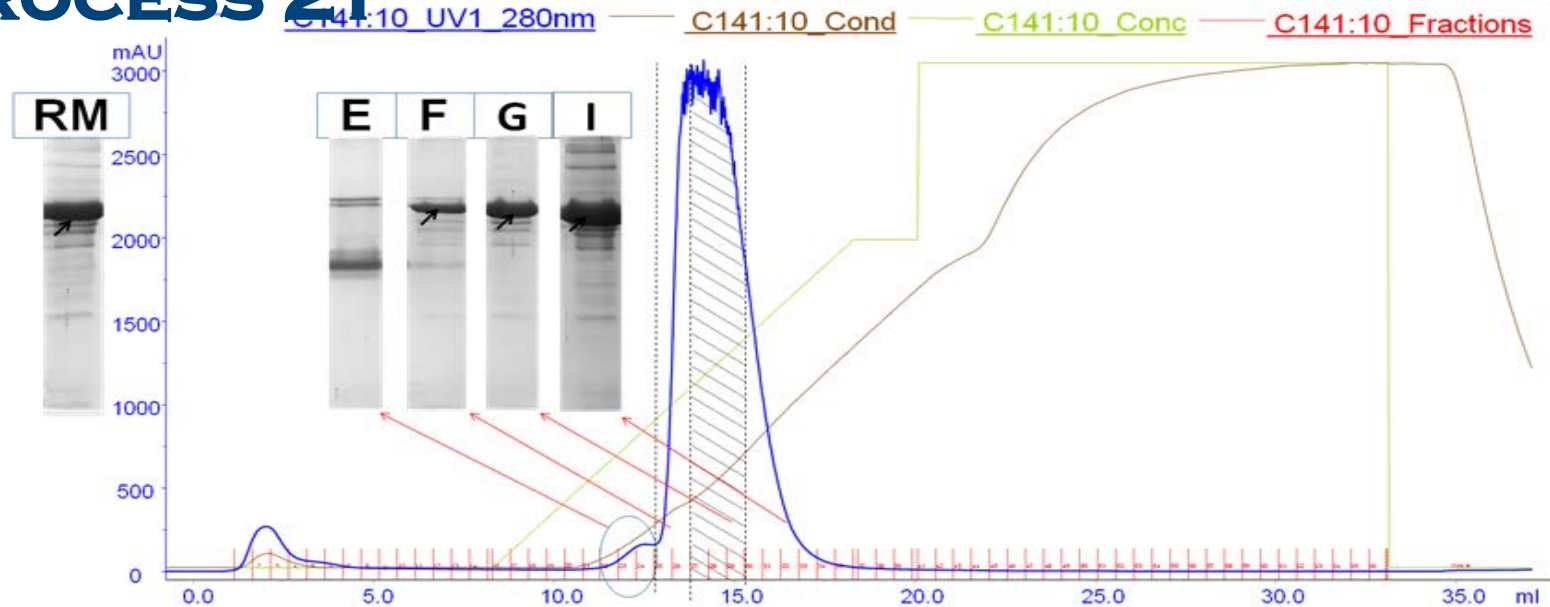


Process selection-

Four different purification processes tested with IEC

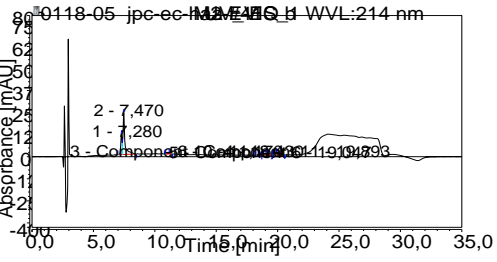
Process N°	HPLC Purity		Yield (gP/IR)	cost (x)
	BSA %	RSD %		
N° 11 BSA	98,82	0,03	63,42	1,00 x
N° 12 BSA	99,10	0,18	65,22	1,50 x
N° 22 BSA	99,25	0,32	20,02	9,91 x
N° 21 BSA	99,13	0,17	39,08	1,01 x

FPLC CHROMATOGRAM OF THE PURIFICATION PROCESS 21



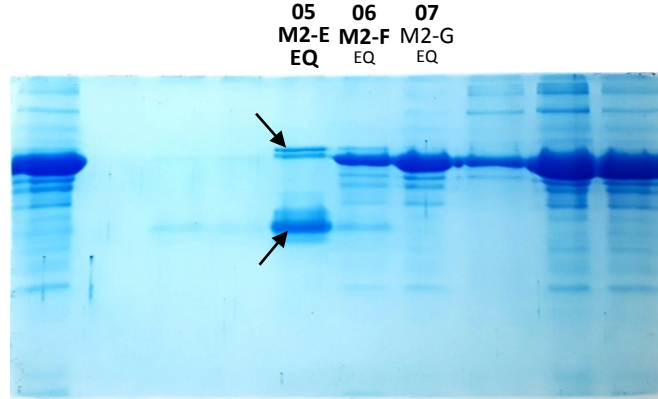
References: R.M.: Raw material. E, F, G (also called BSA Candidate): Purification fractions.
 Black striped area: Fraction selected as CandidateRMc. Black arrow: point BSA on SDS-PAGE profile.
 Blue Graph: Absorbance 280 nm detected. Green Graph: Salt % added for elution.
 Red arrows point: SDS-PAGE profile of the purified fractions. Brown line: Conductivity detected.

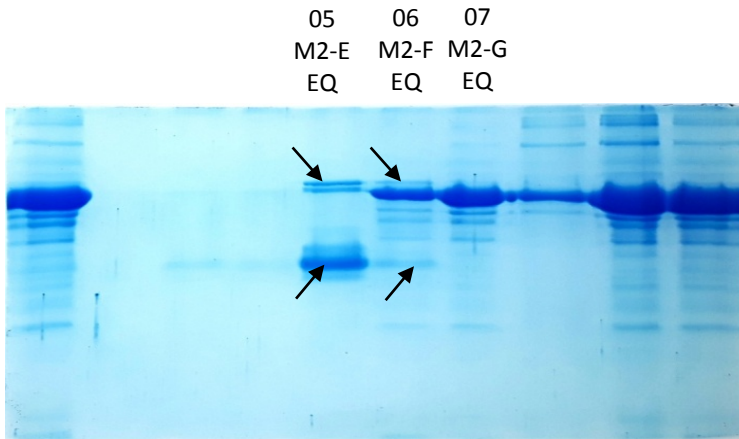
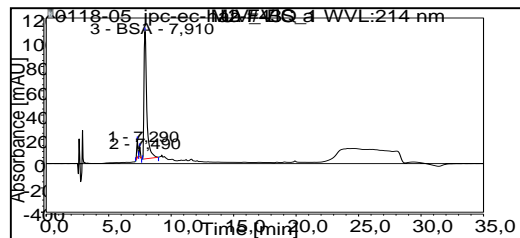
IMPURITIES DETECTED



01
M2-A
EQ

66,2 KDa →

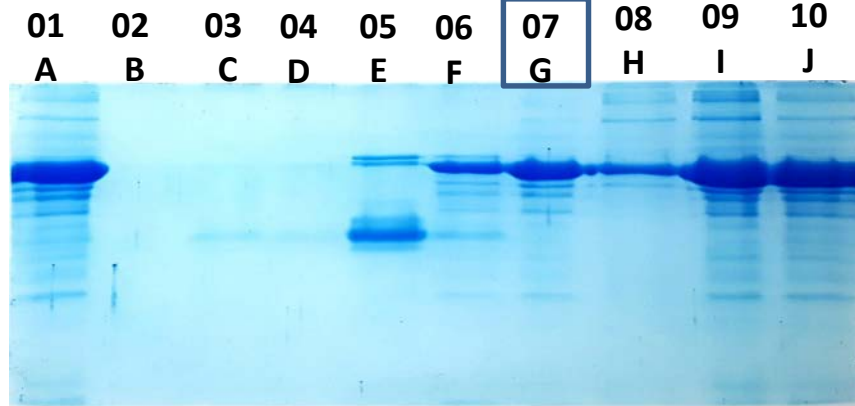
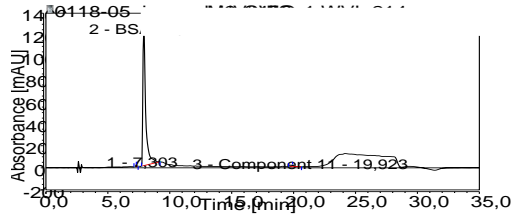




N°	R.T. (min)	Rel. area
1	7,290	5,00
2	7,490	2,62
3	7,910	92,38

PROFILE OF F. "G" LOT# 1

Correlation with production process



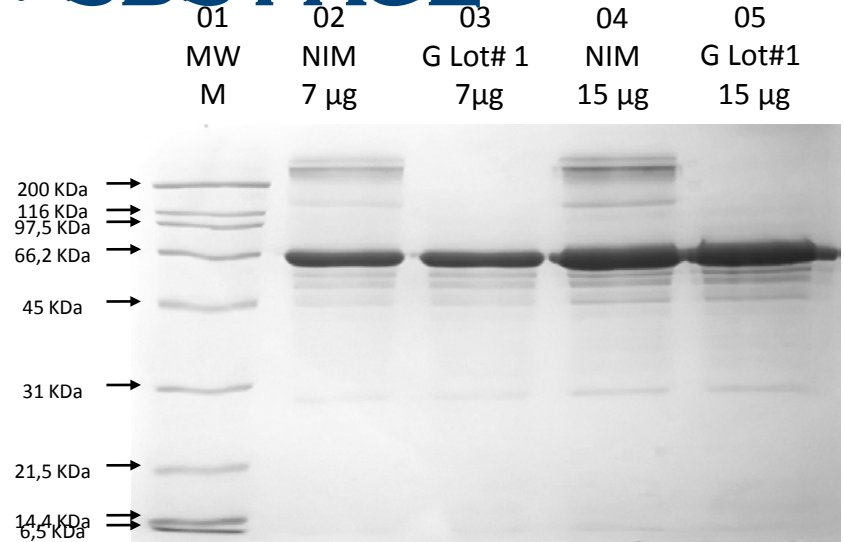
HAS THE PRODUCT BEEN VERIFIED? SDS-PAGE

Sample	HPLC-UV Purity	
	BSA %	RSD%
R.M.2	97,73	1,72
Cand. (LOT#1)	99,13	0,17
Cand. (LOT#2)	99,23	0,13
BSA-CRM NIM	99,26	0,01

EMPAI & DDA

Samples	MS Purity		
	BSA %	RSD%	O.P.P
G Lot# 1	99,49	0,02	28
BSA-CRM	99,96	0,01	13

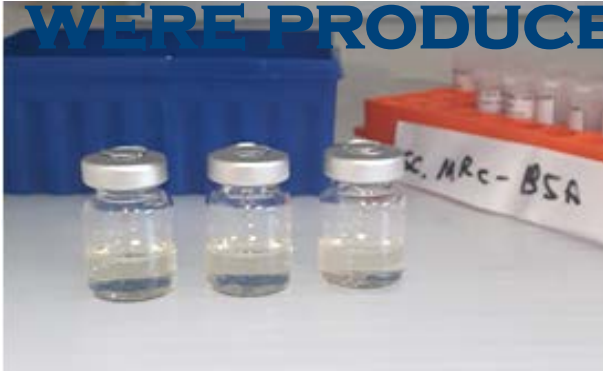
These results were provided by University of Buenos Aires.



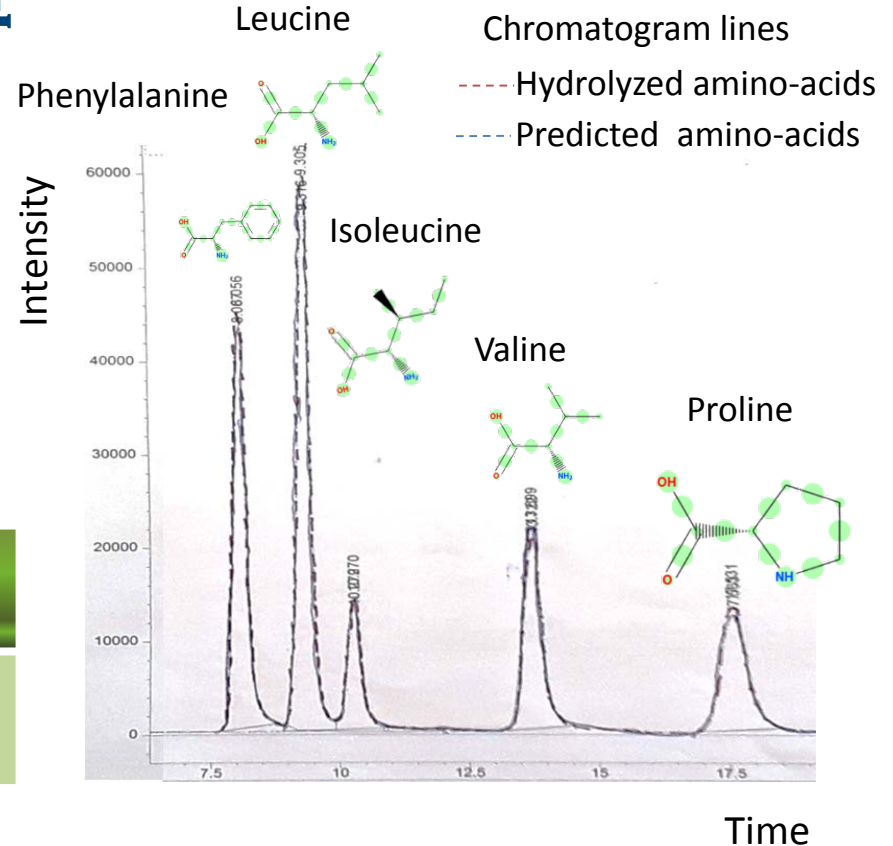
Ref. SDS-PAGE 12%, dried with colloidal coomassie. 01: molecular weigh marker, 02,04: BSA CRM NIM 7 and 15 µg per well respectively, 03 and 05: Fraction G lot#1 7 and 15µg per well respectively.

162 MG BSA CANDIDATE

WERE PRODUCED

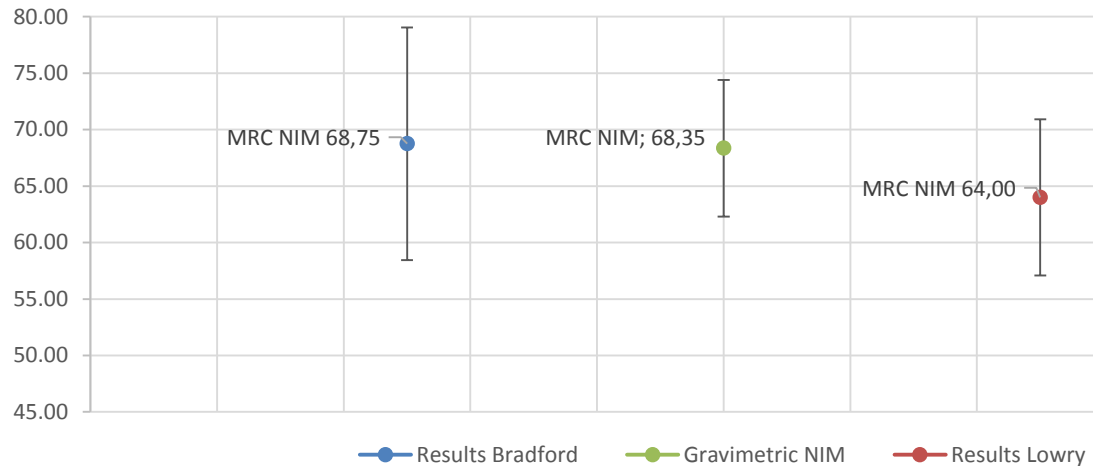


Sample	Reference Value	Exp. Uncertainty
BSA candidate	17,98 mg/g	2.9 %



DOES THIS RESULT WORK?

Colorimetric methods -calibrated with BSA Candidate-



Number of analysis: 6 times.
uncertainty: Intermediate
Precision ongoing.
Does it matter to check if this results are working? I believe that no.

ONGOING PROJECT

• **SCALE-UP PRODUCTION:** INTI will produce 1 lot of 1000 vials, each one with 2 ml of BSA 7% solution, 150 mg of protein each vial.

BSA 7% solution as CRM (under development)



• **CHARACTERIZATION & CERTIFICATION:** will be carried out by NMIs participants



SCALE-UP PRODUCTIO N



20 ml Resin → 0,405 grs.







960 ml Resin → 19,4 grs. (7-9 cycles need to reach 154 gr)

CHARACTERIZATION & CERTIFICATION

● Research

● Certify

Measurement methods	Quantification total protein	Stability study	Homogeneity study
ID-MS (aa- derivatization)	 ●	 ●	
ID-MS	 ●		
HPLC-UV (Purity)			 ●

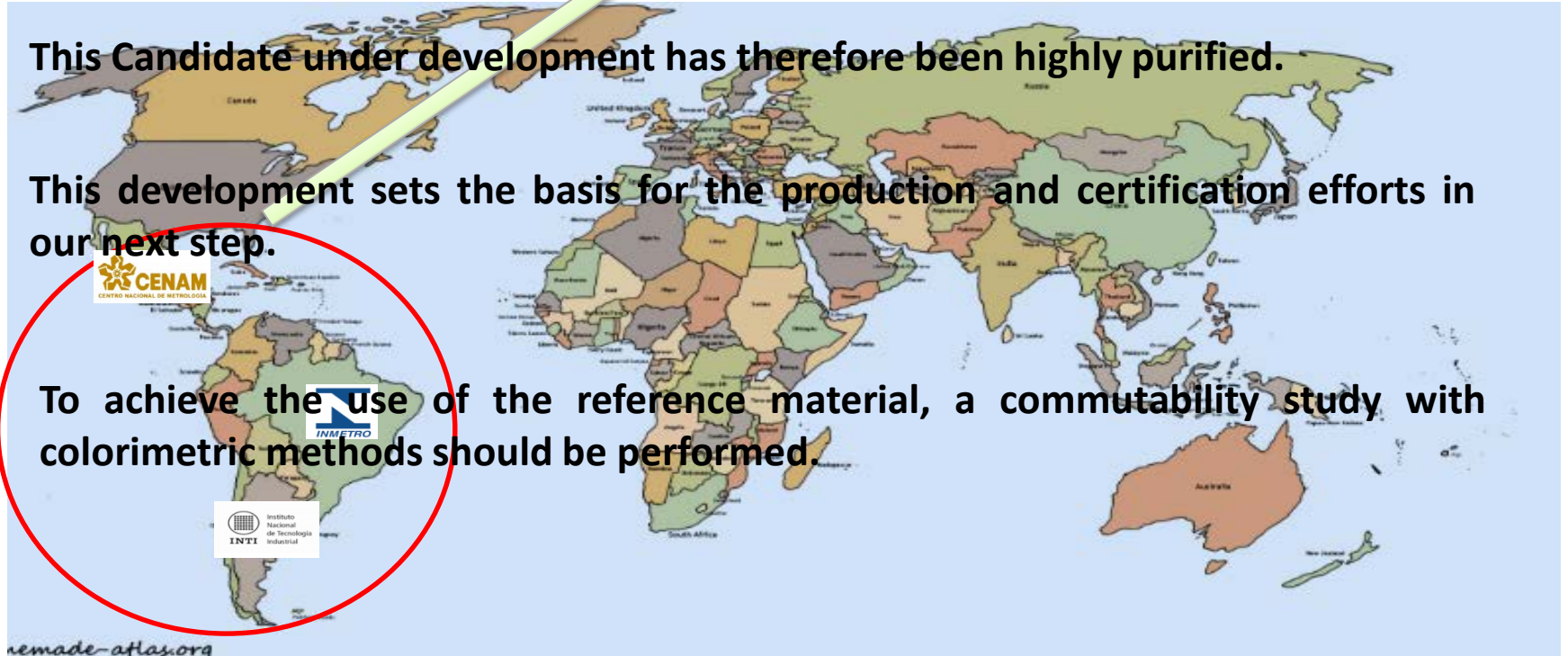
- Mass balance: on lyophilized, INTI
- Purity RMN (INMETRO & INTI)
- Stability study: HPLC-UV (INTI)
- Stability study: Capillar electrophoresis. (INTI)

There is gap in protein RMs production in our region.

This Candidate under development has therefore been highly purified.

This development sets the basis for the production and certification efforts in our next step.

To achieve the use of the reference material, a commutability study with colorimetric methods should be performed.



Acknowledgements

H. Laiz from SIM P. Gatti & M.A. Cappa, Biotechnology Pilot plant team from INTI. Thank you for your support on the production.

A. Henrion, R. Ohlendorf, C. Arsene, G. O`Connor from Physikalisch-Technische Bundesanstalt, PTB, thank you for your help on the analytics.

W. Liqing From National metrology Institute from China (NIM) for your help on HPLC-UV and BSA CRM.

Informal inter- comparison

In order to compare amino-acid analysis results (ID-MS), we are interested on performing a comparison between others NMIs, during 2019.



¡Muchas gracias!
Thank you!
Vielen Dank!
Merci!
謝謝！



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BSA COVERAGE

SEQUENCE DETECTED

MKWVTFIS...
 IAFSQYLQCCPFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFG
87.52 %
 DELCKVASI...
 DPNTLCDEFKADEK...
 QECCQAEDKGA...
 KAWSVARLSQK...
 ADLAKYICDNQD...
 ADFAEDKDVCK...
 EATLEECCA...
 EYGFQNALIVR...
 PCTEDYLSLIL...
 TYVPKAFDEKL...
 LKTVMENFVA...
 FVDKCCAADDKEACFAVEGPKLVVSTQTALA



DETECTED FRAGMENTS



NO DETECTED FRAGMENTS



SIGNAL FRAGMENTS NOT INCLUDED IN THE MATURE PROTEIN

SEQUENCE USED FOR BSA QUANTIFICATION BY ISOTOPIC DILUTION

Bottom-up proteomic, HPLC-ESI-Orbitrap:

Hydrolysis: BSA-Trypsin (1:200) over night

HPLC: Gradient acetonitrile:water-TFA 0,1%

Column: Phenomenex C18, 90A.

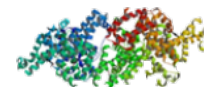
Detector: Orbitrap (resolution 60.000)

Analysis software: Skyline.

Missed cleavages: 0

Precursors charge: 1, 2.

Ion type looked: Precursors.



DEVELOPMENT OF A CRM OF BSA.

APPLICATION



“Strengthening National Metrology Institutes (NMIs) in the Hemisphere, in support of emerging technologies”, 2017.

PROJECT TIMELINE

Jun 2017- July 2019

NMIS PARTICIPATING



FOUNDING



40.000 U\$D.

NETWORKING

- Workshop, “Protein CRM and Bio-metrology”, 27- 29 de June de 2017, Río de Janeiro, Brazil.



- project RG-T2682 IDB, BSA reference material.



- application for second step production further purification and lyophilized BSA CRM development.



- Development of a new proposal for “foot and mouth disease”. DNA reference Material. Fast quantification and detection (Enero 2019). **INTI-INM (Colombia):**



NETWORKING

- Short stay as Scientis guest mass spectrometry. Lic. Hugo Amedei- André Henrión.



- Donation lyophilized BSA CRM, personal quotes from Phd. Wu Liqing.



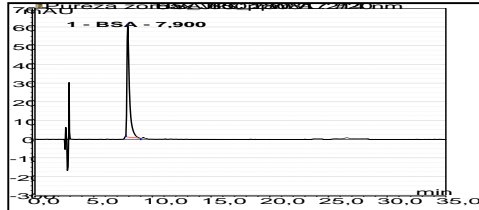
- Personal notes HPLC-UV purity by HPLC-UV.



- Poster & paper to show our work in workshop ""Advances in Metrology in Chemistry and Biology 9-10 April, 2019, Sèvres, France""



600ppm BSA

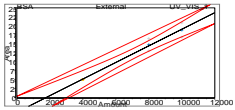


→ Confirmed by NIM

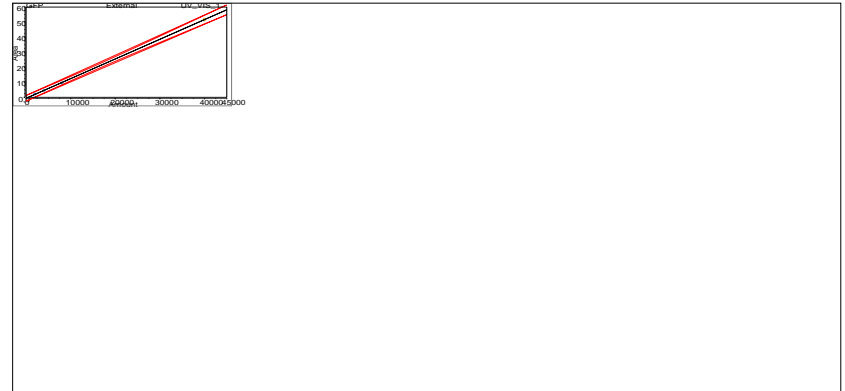


Calibration Line:

$$Y_i = 0,023\text{mAU/ng} * X_i - 41,00\text{mAU.}$$
$$r^2 = 0,9985$$

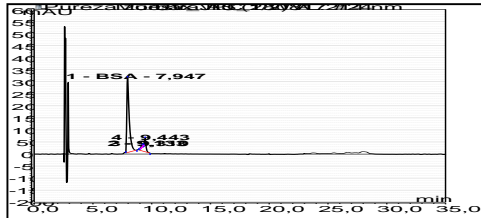


$$Y_i = 0,002\text{mAU/ng} * X_i + 0,245\text{mAU.}$$
$$r^2 = 0,999$$

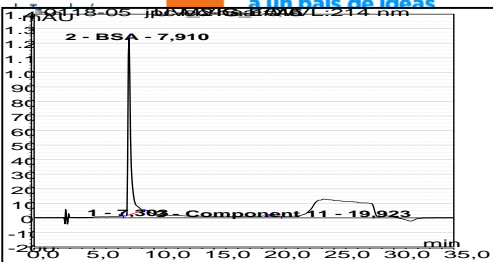


Picture 1: area (Mau*min) vs amout of sample (ng of sample) for BSA (Left) & GFP (right).

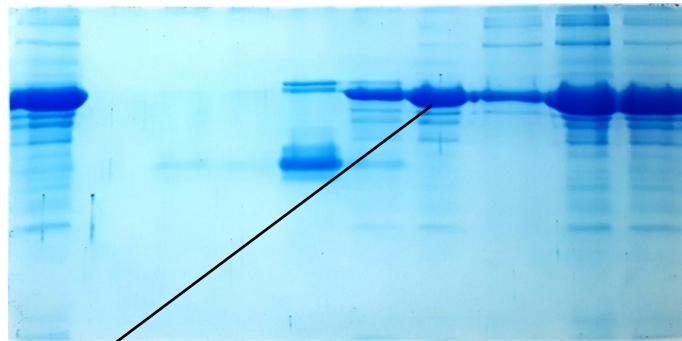
600ppm BSA + 400 ppm de GFP



Confirmed by FLD Detector



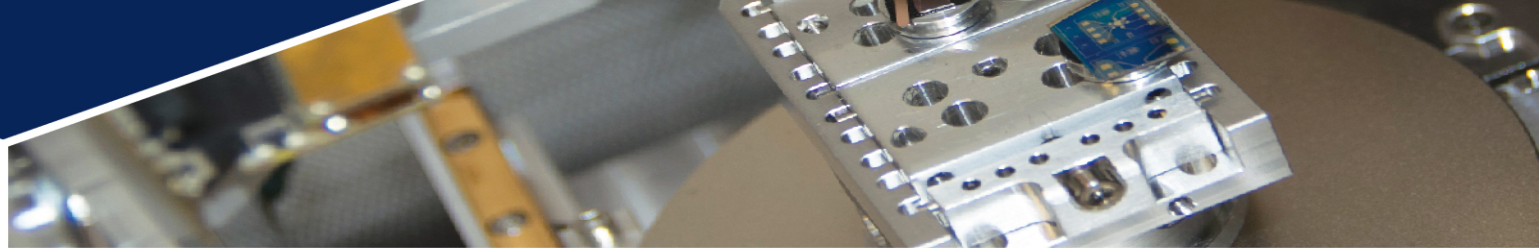
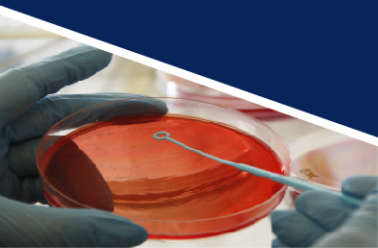
No.	Peak Name	Retention Time min	Relative Area %
1		7,303	0,38
2	BSA	7,910	98,82
3	Component 11	19,923	0,80
Total:			100,00





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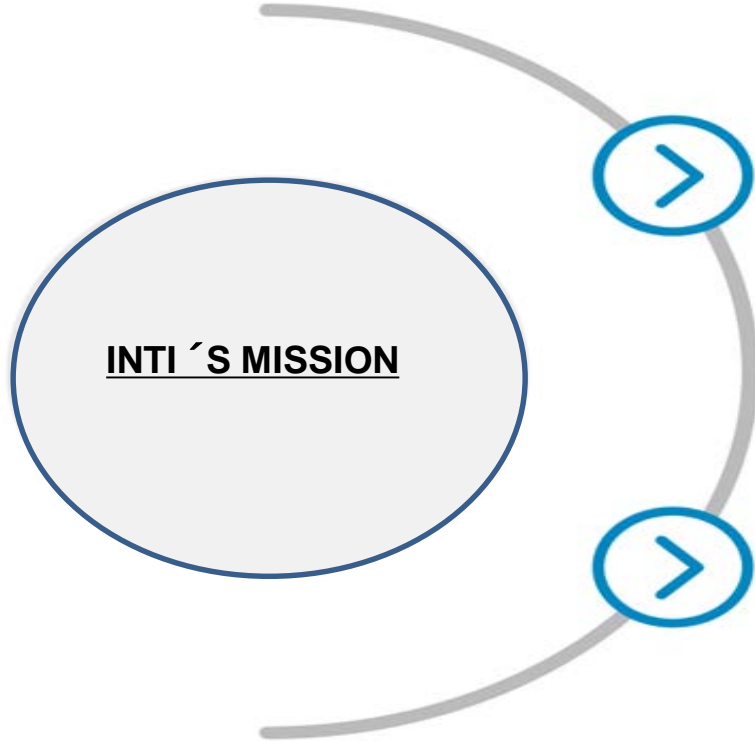
INTI - Biotechnology Pilot Plant- Production Capabilities.

Speaker: MS. Hugo Alejandro Amedei

Workshop : Biometrology, 27-29 June 2017.



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**Promote industrial
development via innovation &
technology transference.**

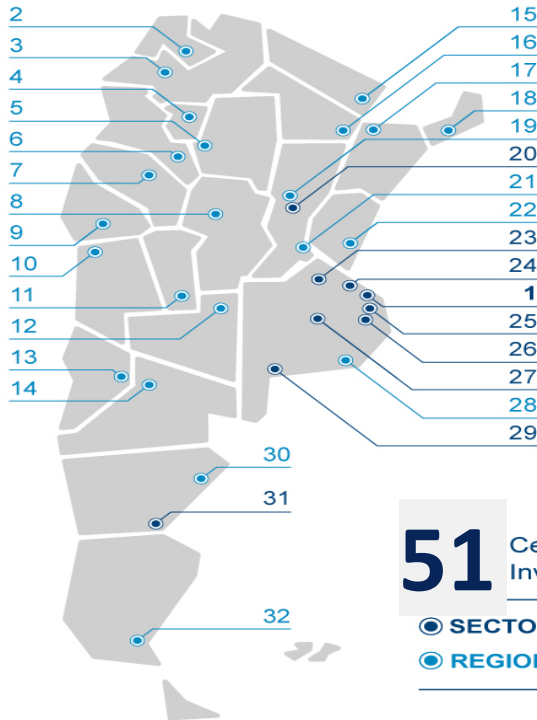
**Strengthen the metrology
capabilities for setting up
traceability & quality of
measurements.**



Strategic Aims

- **Help Industry** to improve productivity, quality, design, process & products.
- **Improve the metrology** in the region.
- We are focused on developing **industries & small business**.

NATIONAL NETWORK FOR TECHNOLOGICAL INNOVATION



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- 32 - Santa Cruz

CERTIFIED REFERENCE MATERIALS AT INTI

Chemistry MRC

Sodium Chloride (For crioscopy)

Methanol-water solution (for Chromatography).

SICECAL



MRC related to Milk

A- D Vitamins

Skim milk and partially Skimmed milk.

Milk powder

Milk powder skimmed

Serum of Cheese

Somatic Cells.














Dulce de Leche (sweet of milk).

REFERENCE MATERIALS AT INTI

- Water-Methanol Solution to **Quantify Methanol by Chromatographic Methods.**
- INTI has **Mass Calibration and Measurement Capabilities (CMCs)** for Quantification of **Methanol/water.**
- Its mass fraction Certified is: $0,500 \% \pm 0,007\%$.



Credit: María Silvina Aued (INTI-Environment, Quality and Metrology).

Measurement methods	Quantification total protein	Stability study	Homogeneity study
HPLC- aa derivatization			
ID-MS			
ICP-MS			
SILVER			
HPLC-UV (Purity)			
Moisture			
Ignition			
Capillary Electrophoresis			

Why 6 was afraid of 7?
Because 7 8 9.



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DEVELOPMENT OF A BSA REFERENCE MATERIAL.

**M.S. HUGO AMEDEI,
PAWG- APRIL 2019.**

Suma valor
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“Strengthening National Metrology Institutes (NMIs) in the Hemisphere, in support of emerging technologies”, 2017.

BSA CERTIFIED REFERENCE MATERIALS

NIST National Institute of
Standards and Technology
U.S. Department of Commerce



BSA 7 % solution , SRM#297

Production of lyophilized BSA CRM