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IMMUNOsurvey™ EQA-IS



ITALIAN NATIONAL PROFICIENCY TESTING (PT) PROGRAM FOR MEDICAL LABORATORIES

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QUALITY ORIENTED PT / EQA PROGRAM

IMMUNOsurvey™ EQA-IS is a PT/EQA program provided by Medical Systems S.p.A (Italy) in collaboration with Care S.r.l. (Italy)

- Medical Systems S.p.A. is a Diagnostic Distributor, existing for 25 years now, with a Quality System Certified to UNI EN ISO 9002 and UNI CEI EN 46002 for the "commercialisation of in vitro diagnostic reagents and systems, pre and post sales assistance and training courses for users of the commercialised systems". Medical Systems S.p.A. is a member of ECLM and EQALM (*Ref. 1- 2*)
- Care S.r.l. is a Manufacturing Company which provides QC materials and statistical elaborations. Certification of the Care Quality System to UNI EN ISO 9001 and UNI CEI EN 46001 is in process

IMMUNOsurvey™ EQA-IS is registered in the EPTIS database (*Ref. 3*) and is **designed and inspired** to **ISO/IEC Guide 43-1:1997** and to the **ILAC-G13:2000 Guidelines**. It has been established in 1997 and since 1998 two samples are distributed four times a year (*Ref. 4 - 6*)

- It provides one of the **broadest range** of **immunoassay evaluation**: 92 parameters overall (**79 analytes and 13 indexes**). It also provides results processing for every method used in the laboratory: in 2001 cycle **100** methods/systems (and **5** softwares for prenatal risk) from more than **50** different manufacturers have been elaborated
- It has a **unique format**: all samples are provided - lyophilized - in a **barcoded sample tube**
- It offers “**esoteric**” **analytes**: i.e. Homocysteine, Troponin I, Osteocalcin, Intact PTH, ACTH, Unconjugated Estriol, SHBG, PAP, anti-TPO & -TG, Vitamin B12, Folates, EPO, IL-2R, IL-6, TNF-alpha, etc and **critical levels**: e.g. 2nd Trimester hCG (10,000-80,000 mIU/mL)
- It provides **ratio parameters**: Free vs total PSA Ratio (F/T PSA), Free Androgen Index = Testosterone/SHBG x 100 (FAI), Free Estradiol Index = Estradiol/SHBG x 1,000 (ESR) and **additional evaluations** such as: **risk indexes**: Trisomy 21, Neural Tube Defects, Trisomy 19 and **clinical interpretation**: F/T PSA, Trisomy 21, Neural Tube Defects, Trisomy 19
- It consists of **10 schemes**: Fertility and Pregnancy, Prenatal Risk, Thyroid, Oncology, Metabolism, Immuno-haematology, Myocardial markers, Allergy, ToRCH & H.pylori serology, Hepatitis B markers (*in development: Clinical Chemistry, Autoimmunity, Urine*)
- It is a **consistent non profit and voluntary** PT/EQA program with a high participation rate: more than 540 Italian laboratories for the year 2001 (64 % Public Labs, 36% Private Labs)
- It benefits from a dedicated **Website** (English: www.eqa-is.org; Italian: www.veq.it) providing updated information and access to an open **Web FORUM** between participants and the PT/EQA staff members



PARTICIPATION TREND

Methods (4th distribution 2001)

| <i>Year</i> | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 |
|-------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| IMMUNOsurvey Schemes (n°) | 1 | 4 | 6 | 8 | 10 | 10 |
| Laboratory Participants (n°) | 56 | 170 | 318 | 476 | 533 | 547 |

PARTICIPANTS

| | <i>pilot</i> | <i>4th distribution</i> | | | | <i>1st distr.</i> |
|---------------------------|--------------|-------------------------|------------|------------|------------|-------------------|
| Fertility | 56 | 131 | 276 | 371 | 403 | 410 |
| Thyroid | | 36 | 243 | 375 | 419 | 427 |
| Oncology | | 41 | 290 | 355 | 401 | 409 |
| Allergy | | 11 | 40 | 59 | 67 | 67 |
| Metabolism | | | 67 | 129 | 170 | 269 |
| ToRCH & H.pyl. | | | 48 | 132 | 162 | 172 |
| Haematology | | | | 141 | 198 | 206 |
| Prenatal Risk | | | | 42 | 55 | 59 |
| Hepatitis B | | | | | 68 | 82 |
| Cardiology | | | | | 34 | 37 |

| | | |
|-----------------------|--------------------------|-------------------------|
| Abbott Architect | Bouty RIA | Immunotech RIA |
| Abbott Axsym | Brahms Kryptor | Incstar RIA |
| Abbott Imx | Byk Liaison | Merck Magia 120 |
| Abbott Tdx | Byk RIA | Meridian Elisa |
| AlfaBiotech Amplia | Care Elisa | Metra Elisa |
| AlfaBiotech Auraflex | Centocor RIA | Nichols Advantage |
| AlfaBiotech Elisa | Chematil Elisa | Nichols RIA |
| Alifax Elisa | Chematil RIA | Nuclear Laser Elisa |
| Alifax RIA | Cis RIA | Olympus AU Systems |
| Bayer ACS:180 | Dade Behering BEP III | Orion Elisa |
| Bayer Centaur | Dade Behering Elisa | Orion RIA |
| Bayer Elisa | Dade Behering Stratus C | Ortho J&J Elisa |
| Bayer IFA | Dade Dimension XL | Ortho J&J RIA |
| Bayer Imm.Turb. | Dade Nefelometr. | Ortho J&J Vitros Eci |
| Bayer Immuno1 | Dade Opus | Pasteur Elisa |
| Bayer Magic Light | Delta Biological Elisa | Pharmacia Autocap |
| Beckman Access | Diamedix Elisa | Pharmacia Delfia |
| Beckman HPLC | DiaSorin Copalis | Pharmacia Elisa |
| Beckman Immage Nef. | DiaSorin Elisa | Pharmacia Unicap |
| Beckman Synchron | DiaSorin RIA | Poiesys Elisa |
| BestTest Rapido | Diesse Elisa | Radim Carla |
| Bioallergy Enea III | Diesse Seramat | Radim Elisa |
| Biochem Allertech | DPC AlaSTAT | Radim RIA |
| Biochem Elisa | DPC IMMULITE | Roche Cobas Core |
| Biochem Labotech | DPC IMMULITE 2000 | Roche Elecsys |
| Biochem RIA | DPC RIA | Roche ES600-700 |
| Biochem SR1 | DRG Elisa | Roche Hitachi Imm.Turb. |
| Biolab TLC | DSL RIA | Roche Modular Imm.Turb. |
| Bioline Western Blot | Endogen Elisa | Serawest Elisa |
| Biomerieux Vidas/mini | Euroimmun Elisa | Shering EIA |
| Biorad Elisa | Eurospital Elisa | Shering RIA |
| Biorad HPLC | Eurospital IFA | Stellar IFA |
| Biorad RIA | Eurospital IgE Fast Plus | Tosoh AIA 1200/600 |
| Bios RIA | Fujirebio RIA | Tosoh AIA 21 |
| Biosystems Imm.Turb. | Genesis Elisa | Trinity Biotech Elisa |
| Bouty Elisa | ICN RIA | |

CONTROL MATERIALS

HUMAN SERUM is the only matrix used for the IMMUNOsurvey EQA-IS in order to minimize differences with clinical samples (*Ref. 7*) Sera are selected from healthy blood donors and commercial normal human sera. They are all stabilized with a preservative standard mixture to prevent bacteriological contamination and are clarified by filtration in a sterile environment up to 0.22µm. An additional heat pretreatment is required only for sera selected for Metabolism scheme to enhance antigen stability

- **SINGLE DONOR SERA** are used in the antibody schemes for Allergy, Thyroid Autoimmunity and Torch & H.Pylori serology (except for IgM positive samples). Single units are selected according to endogenous levels of the antibody and according to a suitable volume to provide these schemes with low sample size (0.3 mL) or limited number of participants (< 150)
- **POOLS** are used for 1 mL samples. They are obtained mixing single units, tested for scheme analytes, in order to maintain as far as possible the endogenous concentration (e.g. Fertility scheme: FSH, LH, Testosterone, Progesterone). Current pools are made by an average of 4-5 units. Male or female units are not mixed
- **PATHOLOGICAL SERA** with high titer of specific analytes from commercial sources are occasionally added to the pools to reach a significant target level (e.g. PSA, Toxo IgM and Rubella IgM)

SPIKED ANALYTES are occasionally added to the pools when endogenous concentration is not suitable for a representative control level. Spiked materials are only commercial human antigens derived from extractive and high purification processes or synthetic preparations like chemical, recombinant or bioactive peptides. When possible sample commutability is investigated. Recently, an independent study has confirmed commutability for CEA in samples of the IMMUNOsurvey™ EQA-IS Oncology scheme (*Ref. 8*)

MANUFACTURING PROCESS Sample preparation is based on human matrix enriched, when requested, with concentrated antigen to reach a fixed target level. The liquid homogeneous solution (stirred) is aliquoted into polypropylene vials (16 x 100 or 12 x 75 mm) in the proper volume format (1 mL or 0.3 mL) and lyophilized. A random check of the accuracy of the dispensed volume in each vial is performed to ensure distribution uniformity

QUALITY CONTROL A post manufacturing analytical QC (IQC) is performed to release each batch/lot produced. Vials are controlled for the physical appearance of lyophilized and reconstituted form. All the analytes of the scheme are checked for the following parameters:

- 1) observed vs expected target dose within an acceptability range;
- 2) vial to vial variability;
- 3) natural and accelerated stability for lyophilized and reconstituted samples



Data input: all returned data are elaborated. Due to pitfalls of Outlier tests (*Ref. 9 - 10*) extreme results reported are checked with participants and are deleted only when a technical reason for their aberrant behaviour can be found (*Ref. 11*)

Statistical Analysis is performed according to a statistical procedure elaborated by the provider (i.e. Mean, CV%, Minimum Value, Maximum Value), for both **All methods** and **Method group**.

Assigned values are Analytical Reference value (*Ref. 12*) derived from participants consensus: i.e. **All methods Mean** and **Method group Mean**.

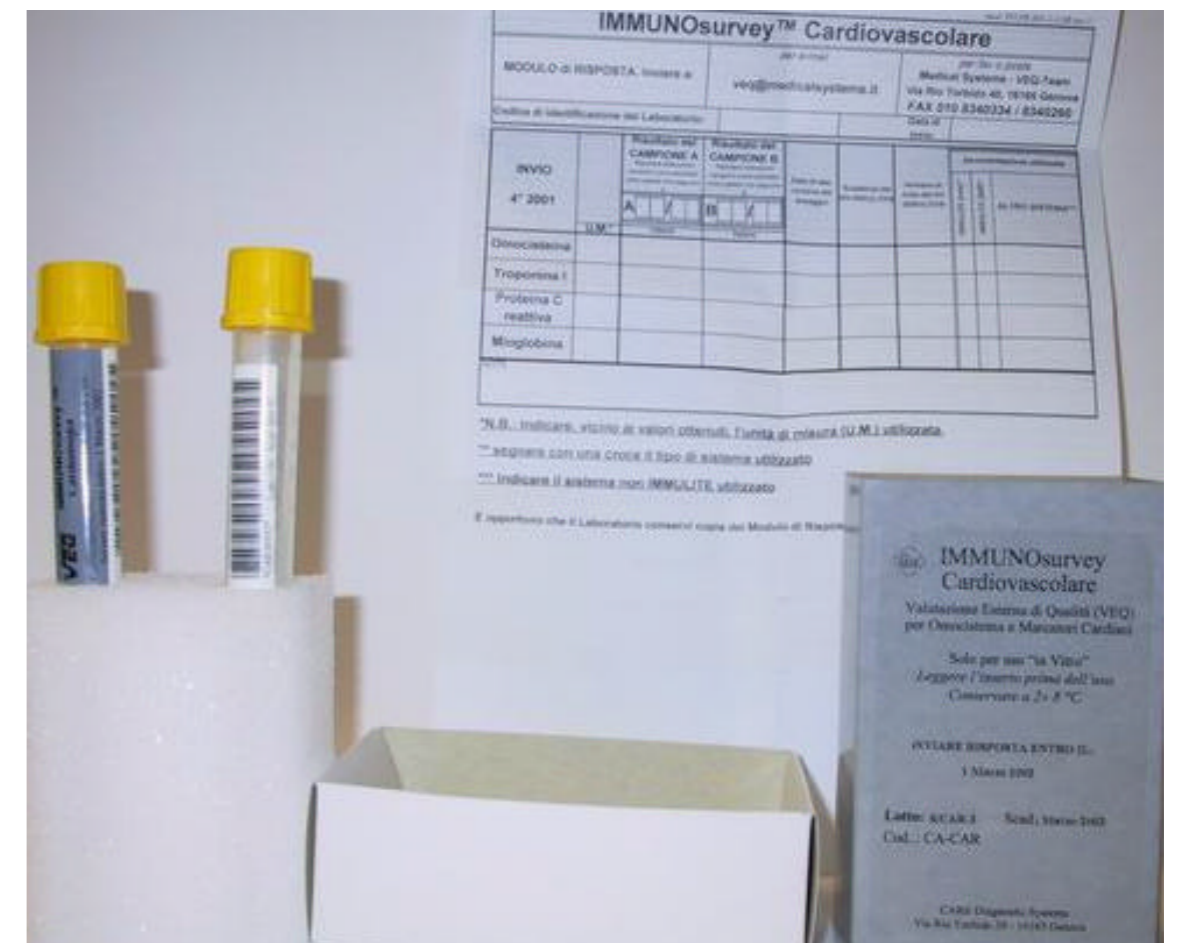
Laboratory performance: for every distribution participants results are pointed out as two **Bias** values: versus **All methods Mean** and versus **Method group Mean** (*Fig. 1*). At the end of the annual cycle a **Cumulative assessment** is performed (*Fig.2*), i.e: **Mean Bias** and **Var Bias**, (Variability of the Bias, 1 SD of all the Bias in the cycle distribution), based on the deviation from both **All methods Mean** and **Method group Mean**.

Cumulative assessment: the criteria for a Satisfactory participation are given on the basis of values out of the provider experience (*Ref. 4 - 6*).

Mean Bias and **Var Bias** (both versus All participants Mean and also versus the Method group Mean) are used in the performance evaluation. The performance is defined as Satisfactory, if it satisfies the following limits: Mean Bias $\pm 15\%$ and Var Bias $<15\%$.

Scores of Performance (*Fig. 2*) are used in the assessment by growing deviation from the analytical reference value (*Ref. 5*):

1. Satisfactory Mean Bias ($\pm 15\%$) with Satisfactory Var Bias ($<15\%$)
2. Satisfactory Mean Bias ($\pm 15\%$) with Unsatisfactory Var Bias ($>15\%$)
3. Unsatisfactory Mean Bias ($> \pm 15\%$) with Satisfactory Var Bias ($<15\%$)
4. Unsatisfactory Mean Bias ($> \pm 15\%$) with Unsatisfactory Var Bias ($<15\%$)



PERIODICAL REPORT

Four weeks after results receipt, the provider releases to each participant an individual and confidential Periodical Report about the distribution outcome of the PT/EQA schemes.

According to ISO/IEC Guide 43:1997 and ILAC-G13:2000 Periodical Report (Fig. 1) and Final Report (Fig. 2) both contain Tables and Graphs.

Tables Description:

- ① Analyte, reference unit, round distribution, year of the cycle, number of participants methods
- ② Statistical indexes: Total Results, Mean, Coefficient of Variation (CV%), Min.Value, Max.Value for the Method used and for All methods as well
- ③ Participant own results and Bias (%) vs both Method used Mean and All methods Mean

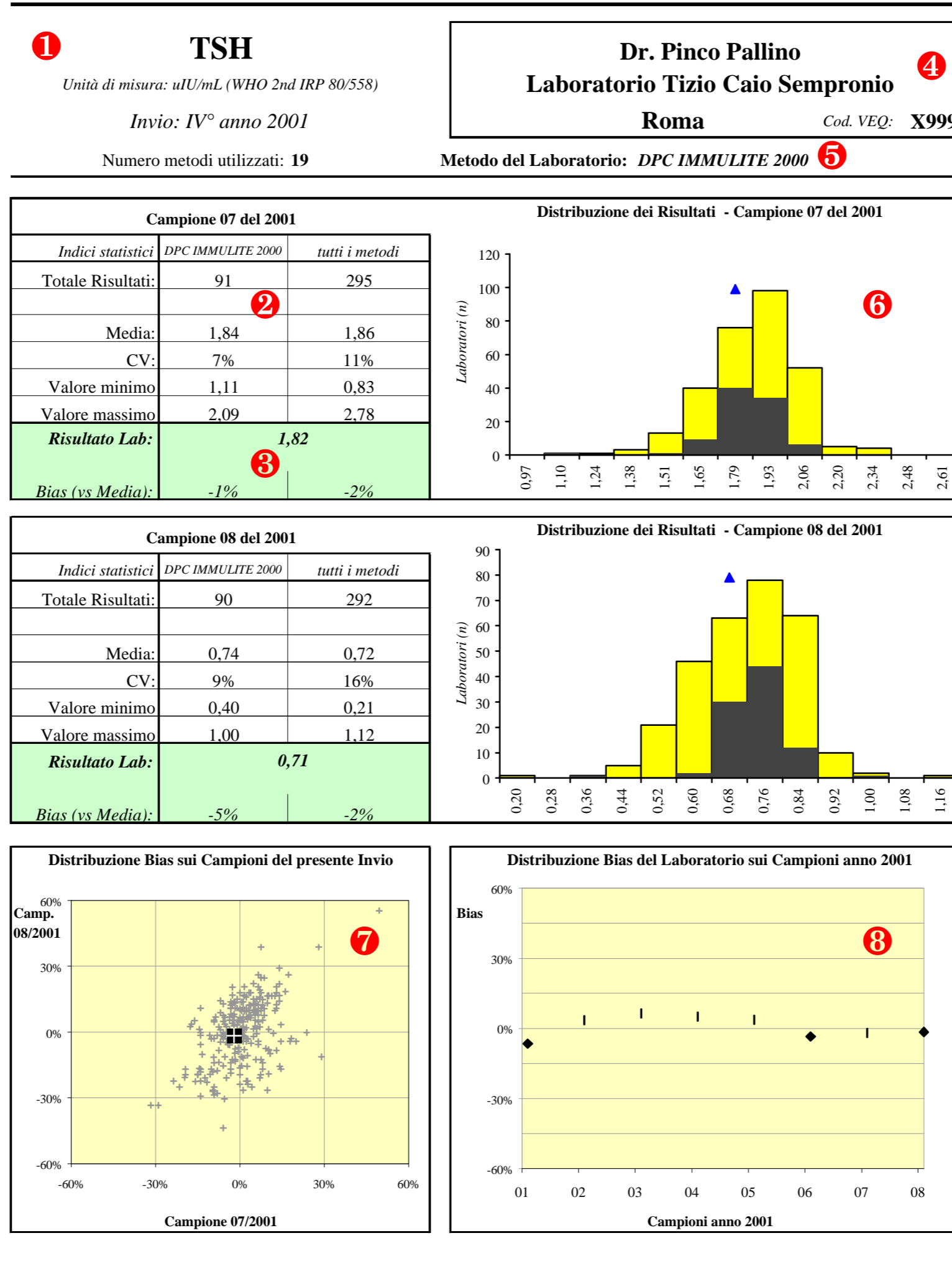


Figure 1

Participants ID:

- ④ Participant reference Name, Laboratory Address, PT/EQA Personal Identification Code
- ⑤ Method / System used

Graphs Description:

- ⑥ Histograms showing the results distribution of both the Method used (Black area) and All methods as well (Yellow area). The laboratory own results are highlighted with a symbol (▲) for visual evaluation
- ⑦ Youden Plot showing pairs of the distribution of the Bias results against All methods Mean for the two evaluated samples in a single figure. The laboratory own results are highlighted (■)
- ⑧ Shewhart Chart with the updated trend of the Participant Bias (|) versus All methods Mean

FINAL REPORT

At the end of the annual cycle participants receive a Final Report for the cumulative assessment of their own parameters in the schemes. Performance assessment is given versus both All methods (1) and Method used (2).

Tables Description:

- 3 Analytes, All participants methods (N°), Lab Var Bias, and Mean Bias, Lab Score, deleted Lab results (N°)
- 4 Analytes, Lab results (N°), Lab Var Bias and Mean Bias, Lab Score, Lab Method/System used

Graphs Description:

- 5 Penalty Box Plot showing pairs of the distribution of Lab Mean Bias against Lab Var Bias. Specific symbol/parameter.
- 6 Satisfactory performance Limit (Score 1)

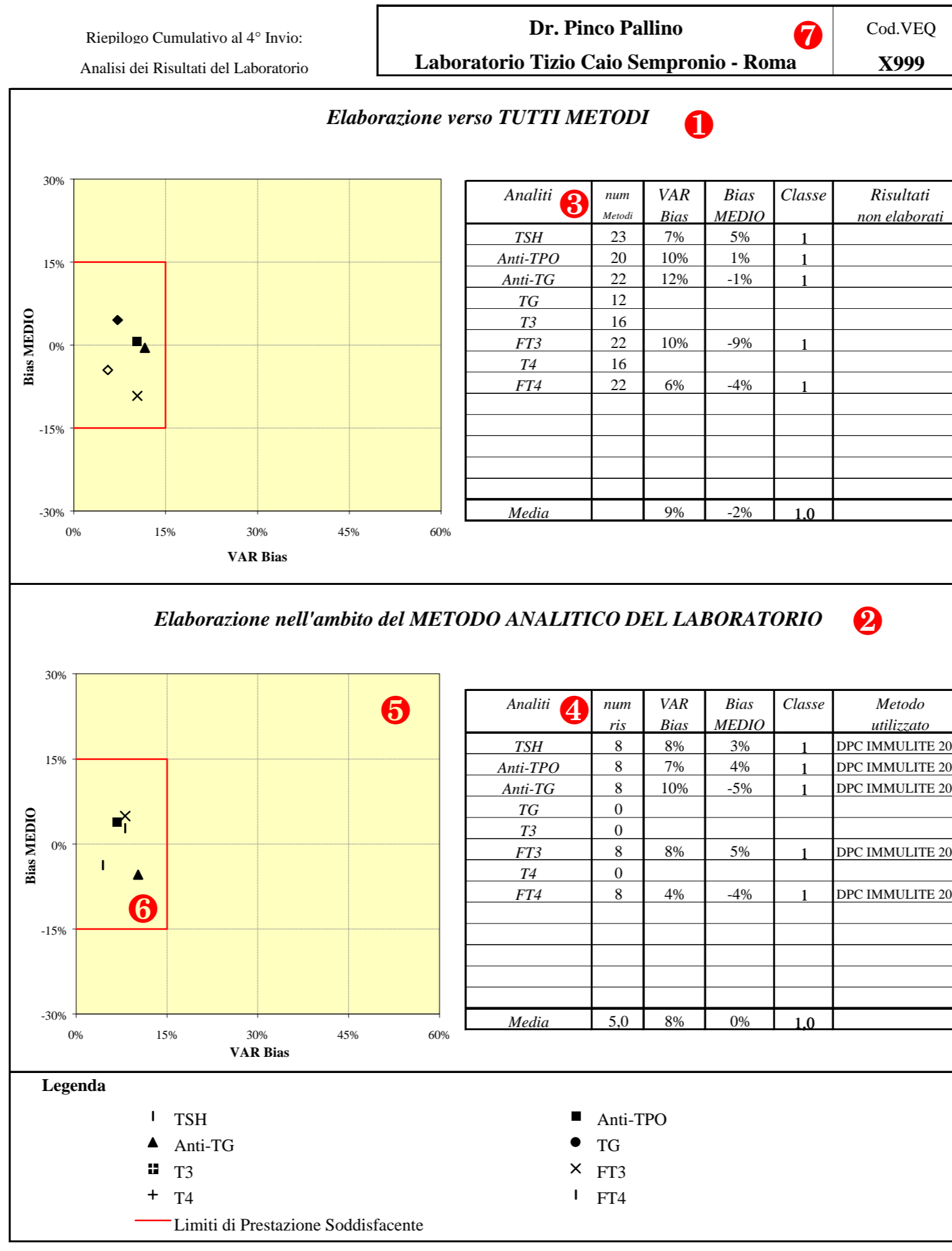
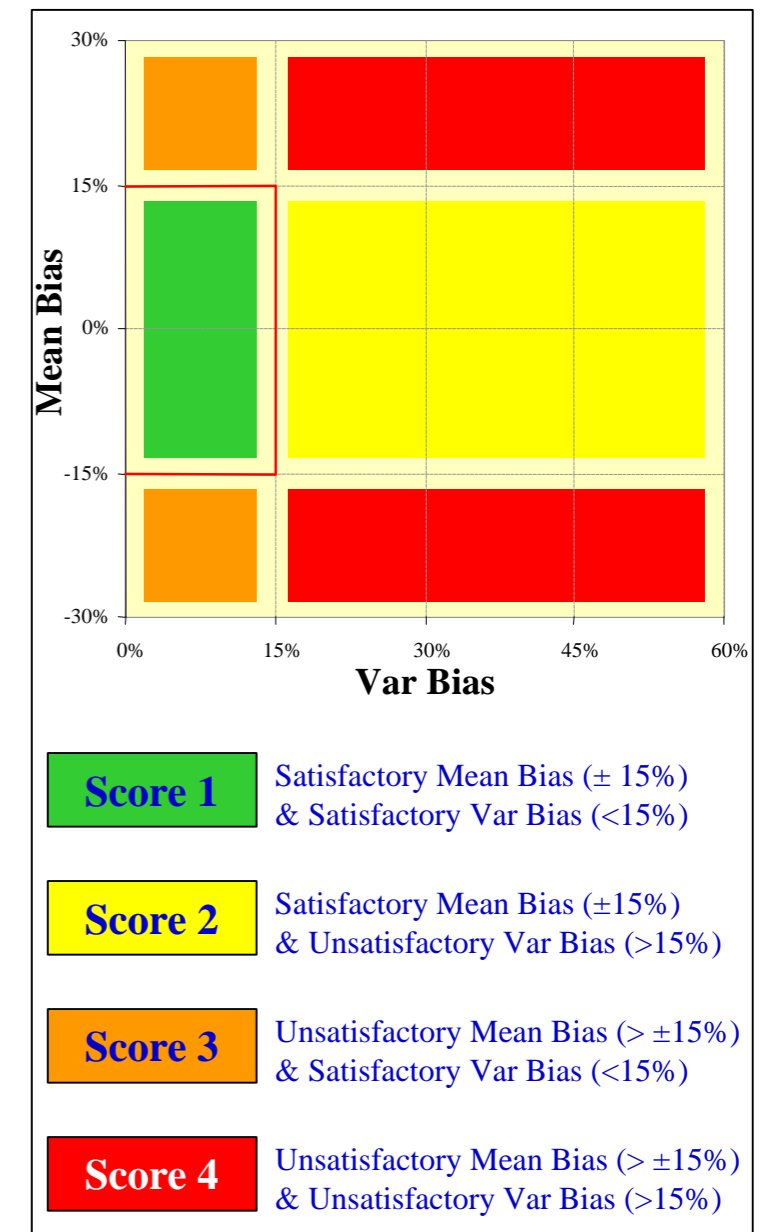


Figure 2

Participants ID:

- 7 Participant reference Name, Laboratory Address, PT/EQA Personal Identification Code

Penalty Box Plot with Score of Performance:



ANNUAL REVIEW - Figure 3

Annual Review Report is the common synthesis of all results shared by and distributed to all participants.

Tables Description (e.g. Metabolism scheme 1):

Parameter evaluated, participating Laboratories (N°) and Methods (N°), results elaborated (N°), average returned results per Lab (N°), Mean CV (%), percentage of participants Laboratories belonging in the 1st Score of Performance (%) and range of samples evaluated

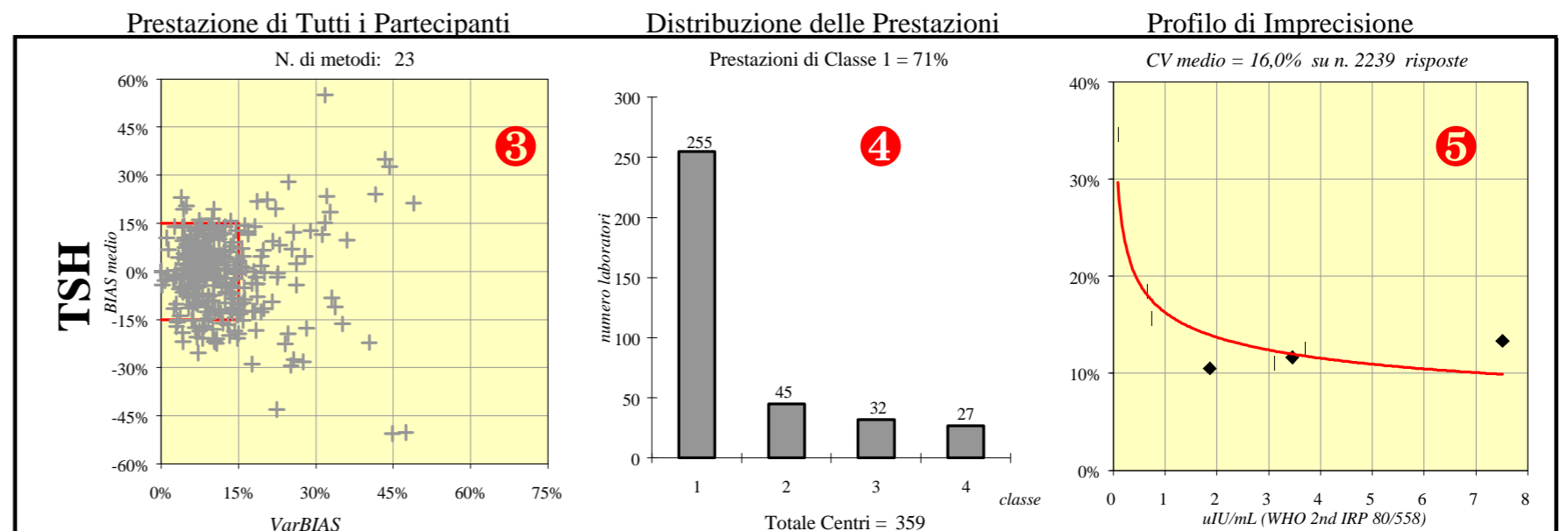
1 Risultati Generali di Tutti i Partecipanti

| Profilo | Analiti | Centri | Metodi Utilizzati | Risultati Elaborati | Media Risultati per Centro | CV Medio | % Centri in I° Classe | Range Campioni |
|-------------|--------------|--------|-------------------|---------------------|----------------------------|----------|-----------------------|----------------|
| Metabolismo | PTH Intatto | 213 | 5 | 1.623 | 7,6 | 29% | 12% | 10,5 - 574,9 |
| | Osteocalcina | 92 | 4 | 441 | 4,8 | 173% | 2% | 15,6 - 50,5 |
| | Insulina | 187 | 9 | 1.317 | 7,0 | 35% | 9% | 76,2 - 219,9 |
| | C-Peptide | 186 | 5 | 1.393 | 7,5 | 15% | 83% | 3,0 - 8,6 |
| | hGH | 121 | 9 | 922 | 7,6 | 20% | 74% | 5,2 - 21,2 |
| | ACTH | 154 | 6 | 1.110 | 7,2 | 23% | 58% | 28,2 - 420,6 |
| | Cortisolo | 206 | 12 | 1.493 | 7,2 | 23% | 72% | 10,5 - 14,9 |

IMMUNOsurvey™ Tiroide

2

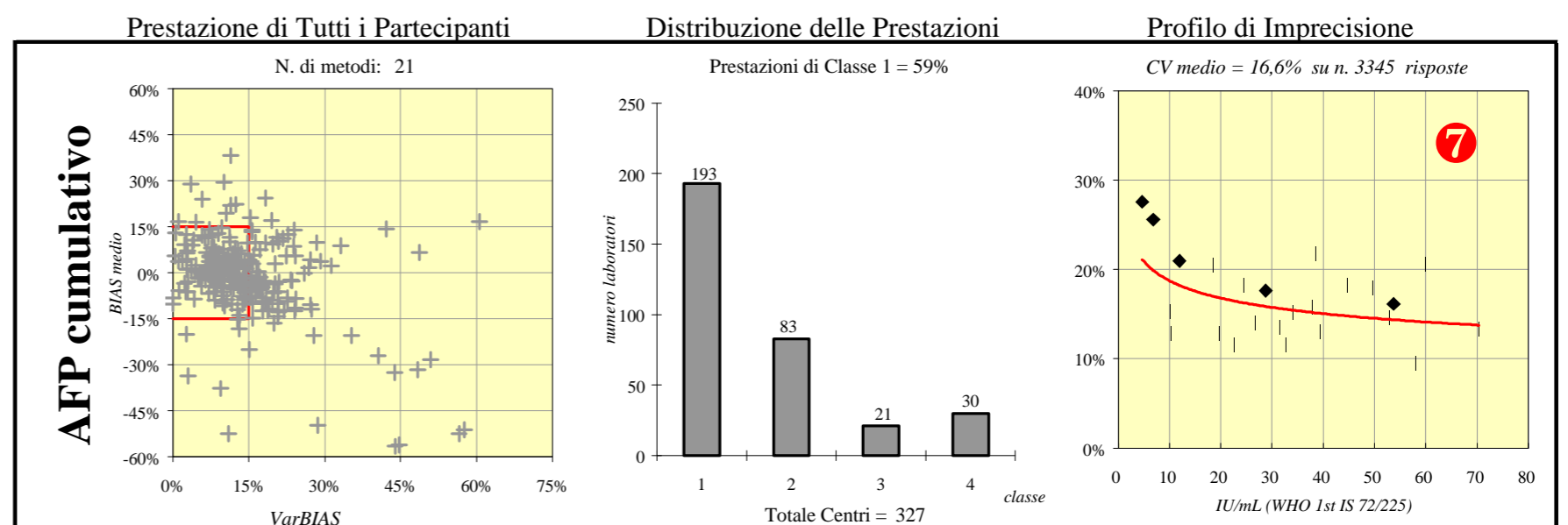
Riepilogo Cumulativo - Ciclo VEQ 2001



IMMUNOsurvey™ AlfaFetoproteina

6

Riepilogo Cumulativo Interprofili - Ciclo VEQ 2001



Graphs Description. For all parameters in each scheme 3 pictures are showed (e.g. TSH, Thyroid scheme 2):

3 All participants Performance. Penalty Box Plot of Mean Bias against Var Bias. Above: All participants methods (N°)

4 All participants Performance distribution within the four Scores. Histograms with Lab (N°) in each Score. Above: All Lab (%) with Score 1. Below: All participants Lab (N°)

5 All participants Imprecision profile for the 8 samples in the annual cycle. Above: Mean CV (%) and all results (N°). Below: range of samples tested, with units and standardization

For parameters evaluated in more schemes (i.e. AFP, hCG, uE3, TG) an Inter-schemes Graph is also supplied (e.g. AFP: Fertility, Oncology and Prenatal Risk 6):

7 Inter-schemes Imprecision profile (24 samples overall)



CONCLUSION & REFERENCES

IMMUNOsurvey™ EQA-IS schemes aim at improving the performance of laboratories by ways of education (*Ref. 13*)

IMMUNOsurvey™ EQA-IS schemes provide surveys in which identical material is distributed to participating laboratories, which measure different analytes using a variety of routine analytical methods. Observations made in surveys allow conclusions on the performance of analytical systems, individual laboratories, and group of laboratories as a whole

IMMUNOsurvey™ EQA-IS schemes collect information on laboratory measurements, identify possible deficiencies in laboratory practice or for particular methods, materials and equipment under routine. They alert participants about problems related to harmonisation of results and for any corrective action to be taken for improvement

IMMUNOsurvey™ EQA-IS schemes are effective means for the assessment of performance and for post-marketing surveillance of laboratory technology. They can give critical information to laboratories for actions to be taken to improve patient care and diseases monitoring

In conclusion **IMMUNOsurvey™ EQA-IS** program has been found useful, as it initiates a controlled process towards solving technical and organizing problems to improve the quality of service from each individual laboratory as well as to achieve results comparability among laboratories

1. European Confederation of Laboratory Medicine (ECLM). <http://www.inserm.fr/eclm>
2. European Committee for External Quality Assessment Programmes in Laboratory Medicine (EQALM). <http://www.eqalm.org>
3. European Proficiency Testing Information System (EPTIS), supported by the European co-operation for Accreditation (EA), EUROLAB, EURACHEM and sponsored by the European Commission. <http://www.eptis.bam.de>
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