

Which analytic qualities are essential in the interpretation of semen analyses?

Jose Antonio Castilla

**U. Reproduction
HU Virgen de las Nieves
Granada, Spain**

And

**Sperm Bank CEIFER
Granada, Spain**



Slide 1

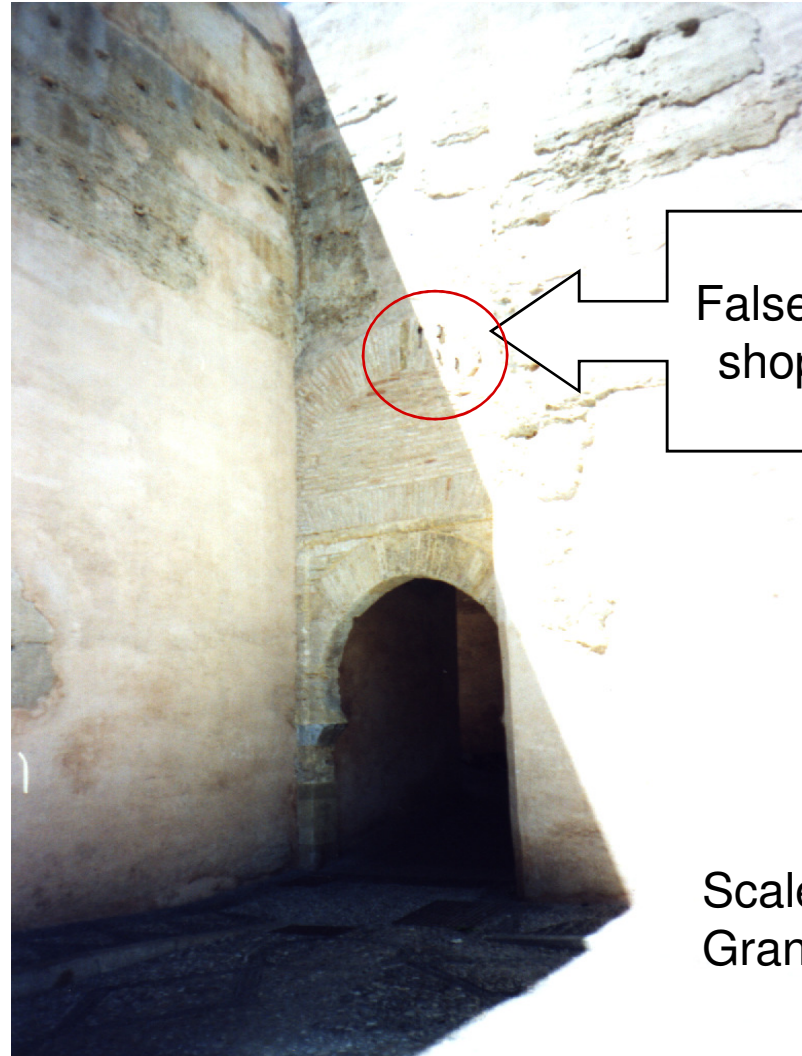
W17 Granada is best known for the Alhambra and the Albayzin.

And also of course for Tapas the wonderful free food you get just for drinking alcohol.

AlbayCF; 15/09/2009

Which analytic qualities are essential in the interpretation of semen analyses?

Tradition of quality control in Granada, Spain



False scale of shopkeeper

Scale gate (IXth Century)
Granada, Spain


Slide 2

W11

we in granada have been interested in analytical quality controls for over 1000 years. As you can see here, in the old quarter of Granada "albayzin", we can find the scale gate, close to the medieval market square. In those days, local officials used to carry out quality controls of shopkeepers scales, and when they found a dishonest shopkeeper trying to cheat his customers by using false weights, they would put the scales above the gate, along with the shopkeepers hands.

Since then we have improved our practices with quality control but we still have the same passion for quality control. Thus today I will talk about analytical quality specification in analytical laboratories.

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Which analytic qualities are essential in the interpretation of semen analyses?



Maximum allowable error without invalidating the medical usefulness of semen analysis.



Quality specifications

Slide 3

W19

I would like to clarify how we define quality specification

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Quality specifications for seminal parameters

- 1.- Why do we need quality specifications?**
- 2.- Why is internal quality control not enough?
- 3.- Why is the analytical CV important?
- 4.- The Stockholm Consensus Conference on Quality Specifications in Laboratory Medicine,
- 5.- Quality specifications for seminal parameters
- 6.- Other uses of Quality specifications
- 7.- Who should set the quality specifications of seminal parameters?

Slide 4

W20

Today we are going to talk about the following points in detail.

we will deal with,
we will touch on
we will take a look at
we will talk about

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1.- Why do we need quality specifications?

ISO-15189

INTERNATIONAL
STANDARD

ISO
15189

First edition
2003-02-15

**Medical laboratories — Particular
requirements for quality and competence**

*Laboratoires d'analyses de biologie médicale — Exigences particulières
concernant la qualité et la compétence*



Reference number
ISO 15189:2003(E)

© ISO 2003

5.5 Examination procedures

5.5.2 The laboratory shall use only validated procedures ... **The methods and procedures selected for use shall be evaluated and found to give satisfactory results before being used for medical examinations.**

5.5.4 Quality specifications for each procedure used in an examination shall relate to the intended use of that procedure.

Slide 5

W2 fulfill the performance specifications
comply with
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W21 First let's look at why we need quality specifications?
AlbayCF; 15/09/2009

2.- Why is internal quality control not enough?

WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction

FOURTH EDITION



WORLD HEALTH ORGANIZATION

Box 4.1: Determining control limits for the \bar{X} chart

The table below shows the sperm concentrations measured by each of four technicians on the first ten QC samples and the calculation of the mean and the standard deviation of each sample.

Sperm concentration (million/ml)

Sample	1	2	3	4	5	6	7	8	9	10
Technician A	38	35	40	34	38	36	44	43	39	43
Technician B	42	36	42	40	40	40	43	43	46	40
Technician C	38	43	40	51	38	33	39	45	35	39
Technician D	34	36	36	37	36	39	42	43	46	34
Mean	38.0	37.5	39.5	40.5	38.0	37.0	42.0	43.5	41.5	39.0
SD	3.27	3.70	2.52	7.42	1.63	3.16	2.16	1.00	5.45	3.74

Are these CV high or low?

Mean CV= 8.7

CV =100* (DS/Mean)

Slide 6

W22

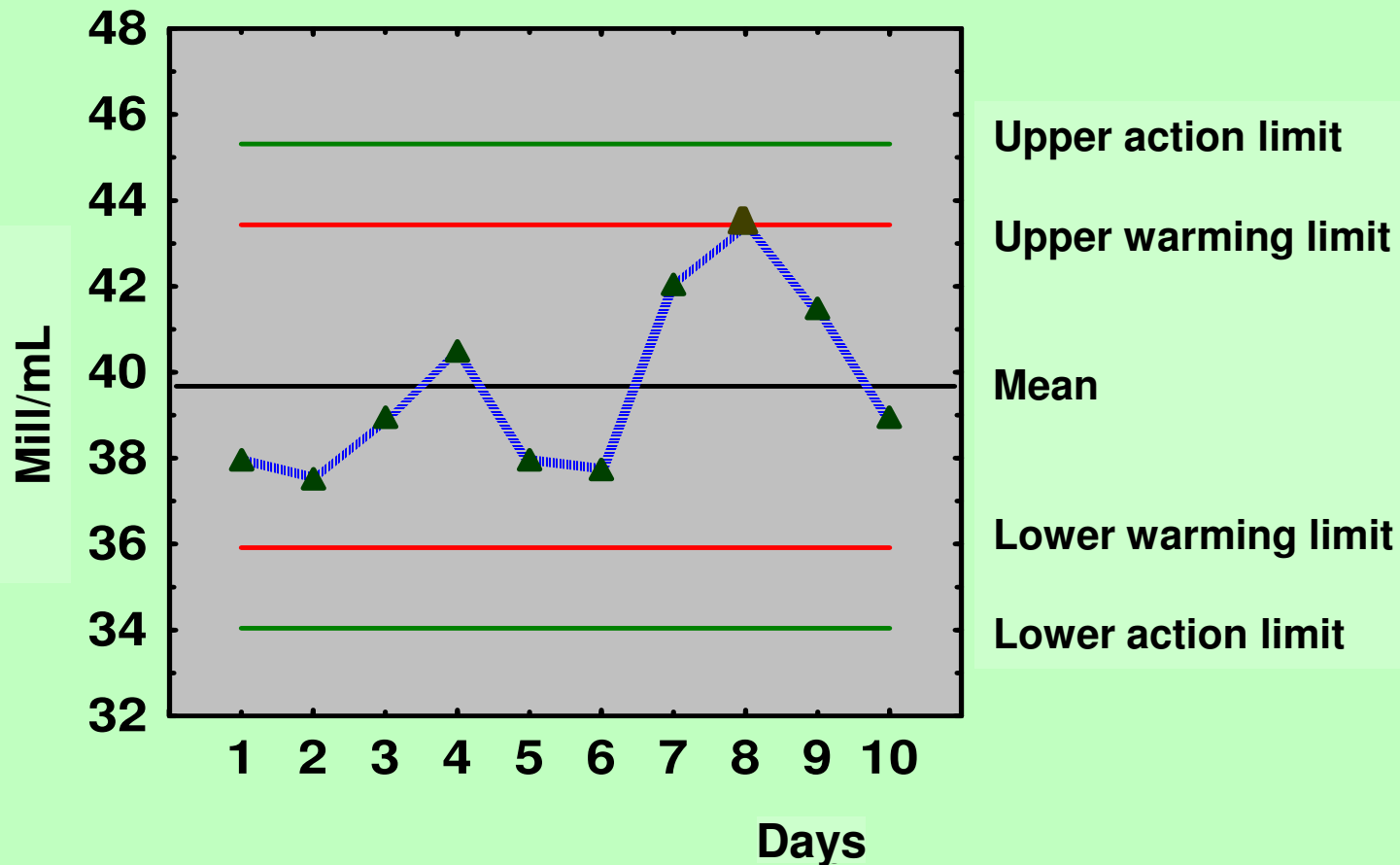
Now why is internal qc not enough?

Taking this table into account, taken from the who 4TH edition we see that

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2.- Why is internal quality control not enough?

Internal quality control: concentration (sperm suspension) **Levy-Jennings \bar{X} Chart**



Slide 7

W3

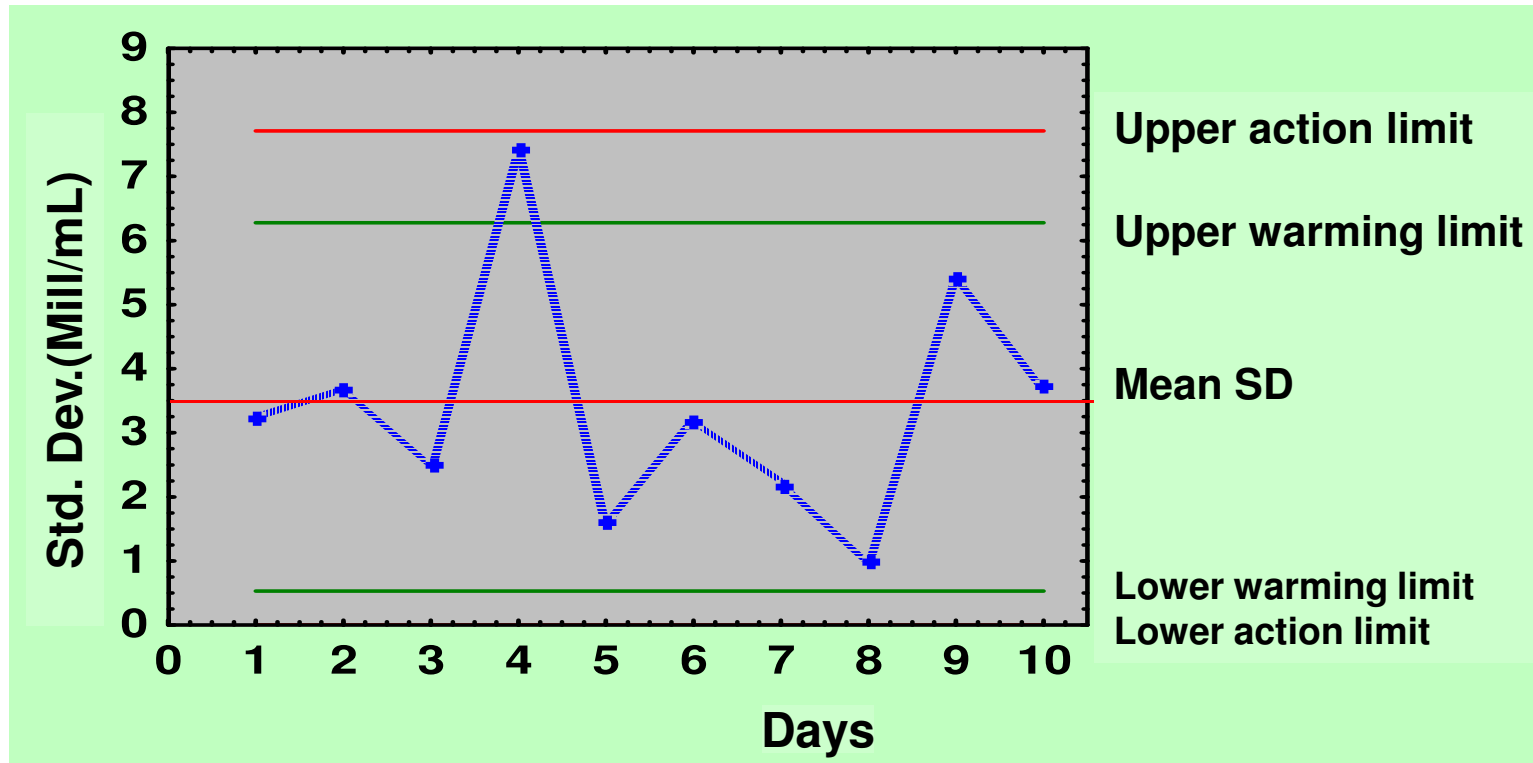
are inbetween the upper and lower warning limits

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2.- Why is internal quality control not enough?

Internal quality control: concentration
(sperm suspension)

Levy-Jennings S_{bar} Chart



Why is internal quality control not enough?

Internal quality control: concentration (sperm suspension)

Technician	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A	38	35	40	34	38	36	44	43	39	43
B	52	36	42	40	40	40	43	43	46	40
C	38	43	40	51	38	33	39	45	35	39
D	24	36	36	37	36	39	42	43	46	34
Mean	38	37	40	40	38	37	42	44	41	39
SD	11.4	3.7	2.5	7.4	1.6	3.1	2.1	1	5.5	3.7
CV	30.8	9.8	6.3	18.3	4.2	9.7	5.1	2.3	13.1	9.5

Slide 9

W23

In theory everything is okay

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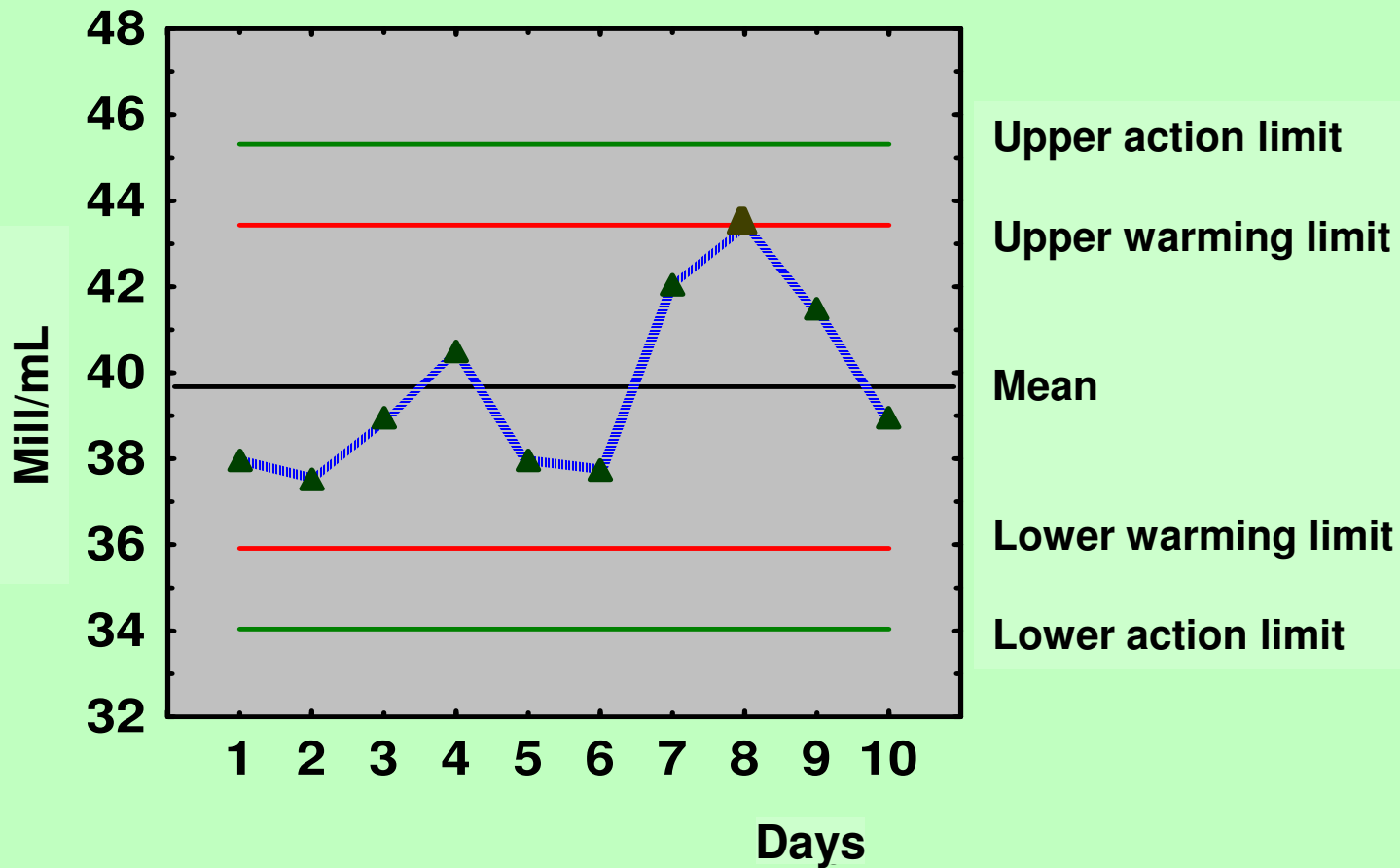
Why is internal quality control not enough?

Internal quality control: concentration (sperm suspension)

Technician	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A	38	25	40	24	38	36	54	33	39	53
B	52	36	52	40	50	50	43	43	56	40
C	38	53	40	61	38	23	29	55	25	39
D	24	36	26	37	26	39	42	43	46	24
Mean	38	37	40	40	38	37	42	44	41	39
SD	11.4	11.6	10.6	15.3	9.8	11.1	10.2	9.0	13.0	11.9
CV	30.8	30.8	26.9	37.9	25.8	30.0	24.3	20.7	31.4	30.4

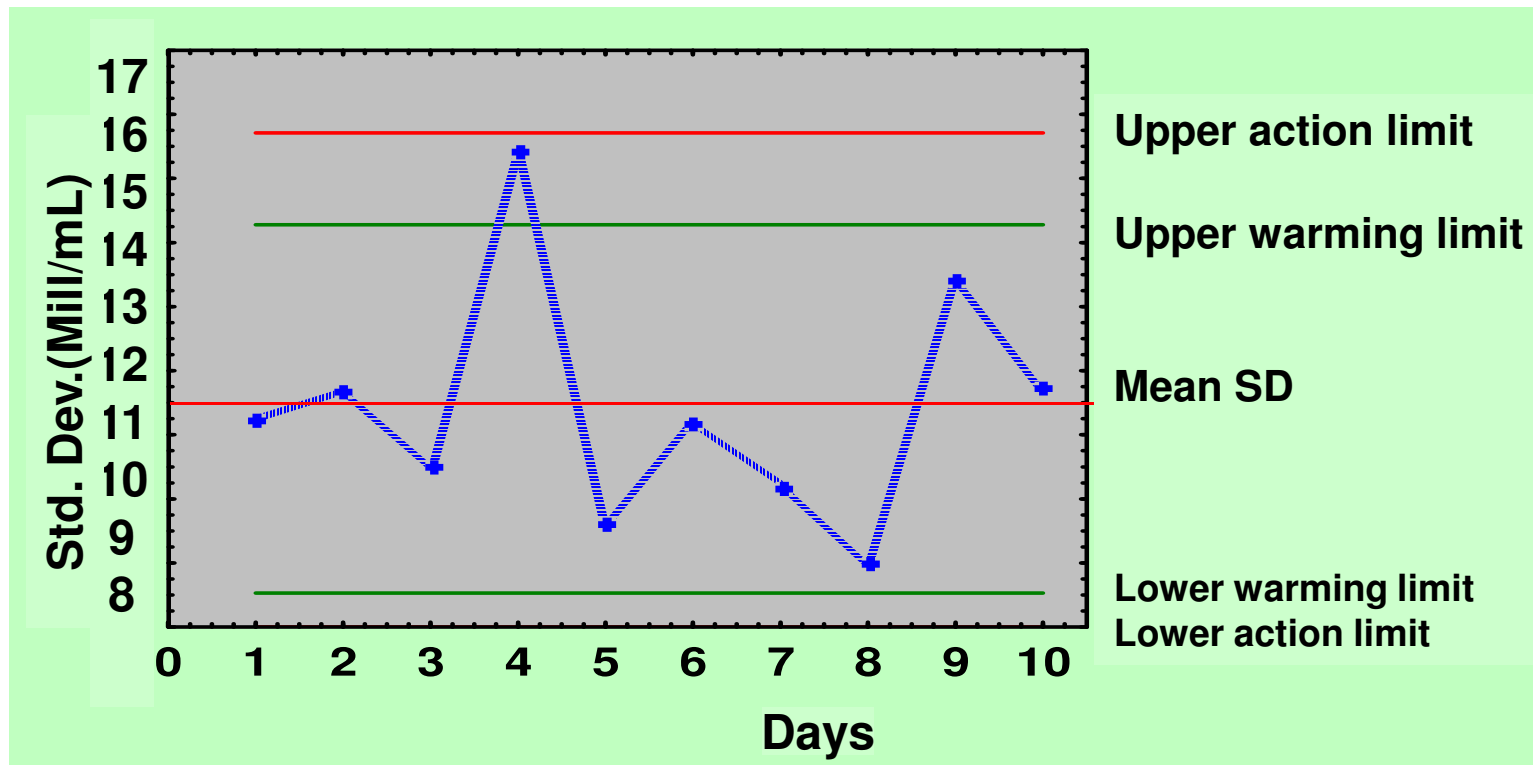
Internal quality control: concentration

Levy-Jennings \bar{X} Chart



Internal quality control: concentration

Levy-Jennings S_{bar} Chart



Slide 12

W36

everything still checks out with the levy jennings chart

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Why is internal quality control not enough?

Internal quality control: concentration (sperm suspension)

Technician	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A	38	25	40	24	38	36	54	33	39	53
B	52	36	52	40	50	50	43	43	56	40
C	38	53	40	61	38	23	29	55	25	39
D	24	36	26	37	26	39	42	43	46	24
Media	38	37	40	40	38	37	42	44	41	39
DS	11.4	11.6	10.6	15.3	9.8	11.1	10.2	9.0	13.0	11.9
CV	30.8	30.8	26.9	37.9	25.8	30.0	24.3	20.7	31.4	30.4

Are these CVs high or low?

Mean CV= **28.9**

CV = 100* (DS/Mean)

Why is internal quality control not enough?

- Same results in Internal quality control
- Only differences in the analytical coefficient of variation

8.7 vs 28.9

- Why is the analytical CV important?

3.- Why is the analytical CV important?

A single analysis of sperm concentration:

The test result is usually reported as a single number, but it represents a range of number, due to:

*analytical random variation –imprecision- C_{va}

*Inherent biological variation –within-subject biological variation - C_{vb}

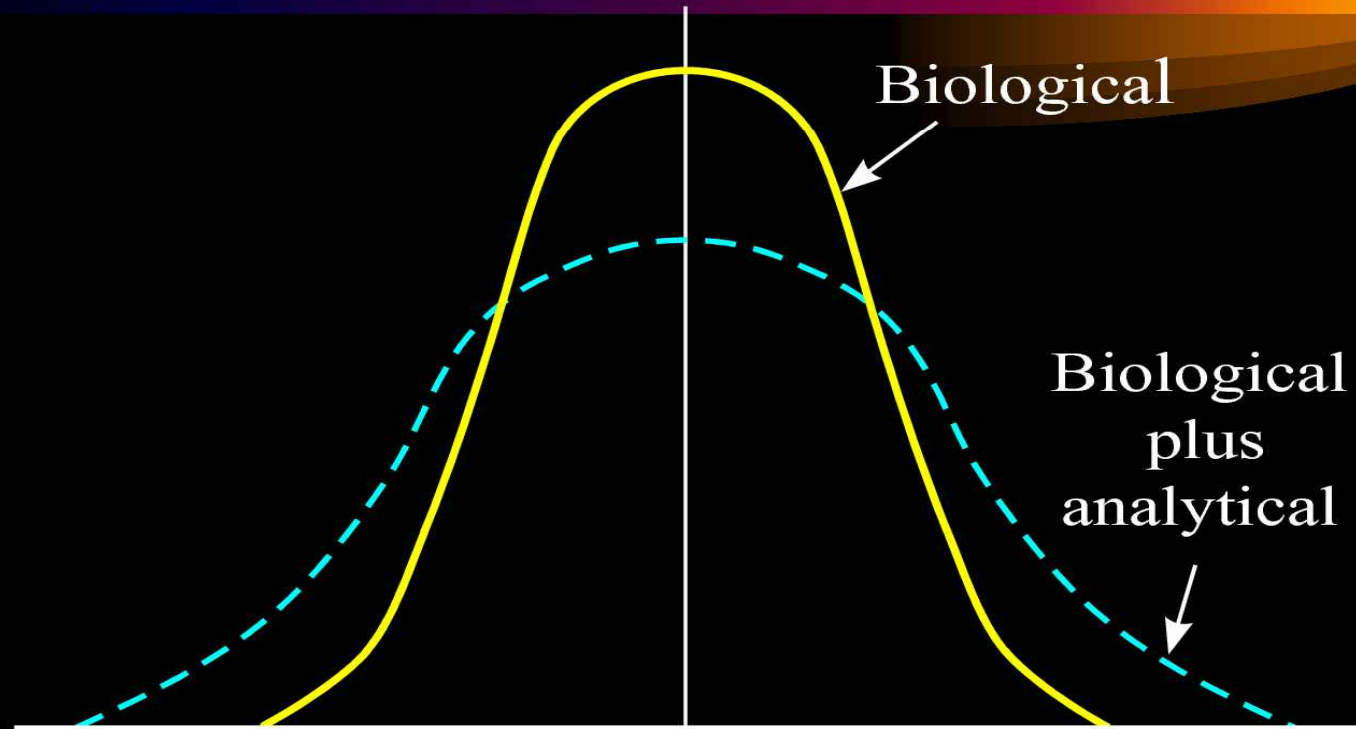
Slide 15

W24

So let's take a look at why the CV is important.

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Analytical imprecision widens inherent biological dispersion

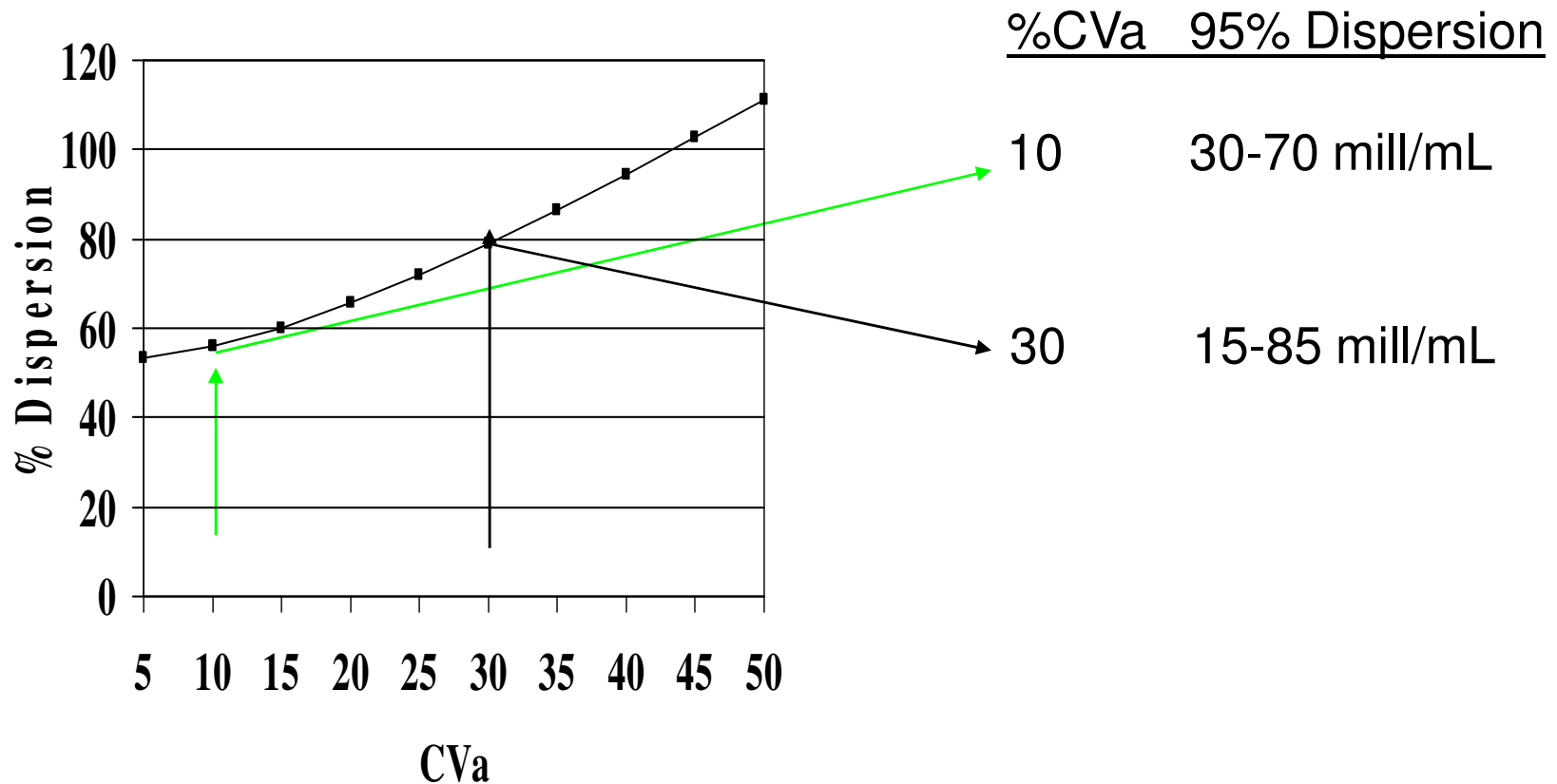


$$\text{Dispersion (\%)} = Z * [(CV_a^2) + (CV_{BW}^2)]^{1/2}$$

Fraser and Harris, 1989

3.- Why is the analytical CV important?

Effect of imprecision on the 95% dispersion of a sperm concentration result of 50 mill/mL (mean of two semen samples)



Castilla et al., Hum Reprod 2006;

Slide 17

W4

Where do we draw the line between acceptable and unacceptable CVa 's? 15%, 20%, 25% where?

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What is the maximum allowable analytical error?

- What are the quality specifications of imprecision in the determination of seminal parameters?

Slide 18

W25

In other words

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4.- The Stockholm Consensus Conference on Quality Specifications in Laboratory Medicine, April 25-26 1999

Hierarchical model to set analytical quality specifications in laboratory medicine (Kenny et al., 1999).



Hierarchical model to set analytical quality specifications in laboratory medicine (Kenny et al., 1999).

1. Evaluation of the effect of analytical performance on clinical outcomes in specific clinical settings

2. Evaluation of the effect of analytical performance on clinical decisions in general:

a. Data based on components of biological variation

b. Data based on analysis of clinicians' opinions

3. Published professional recommendations

a. From national and international expert bodies

b. From expert local groups or individuals

4. Performance goals set by

a. Regulatory bodies (CLIA, ..)

b. Organizers of External Quality Assessment (EQA) schemes

5. Goals based on the current state of the art

a. As demonstrated by data from EQA or Proficiency Testing scheme

b. As found in current publications on methodology.

Slide 20

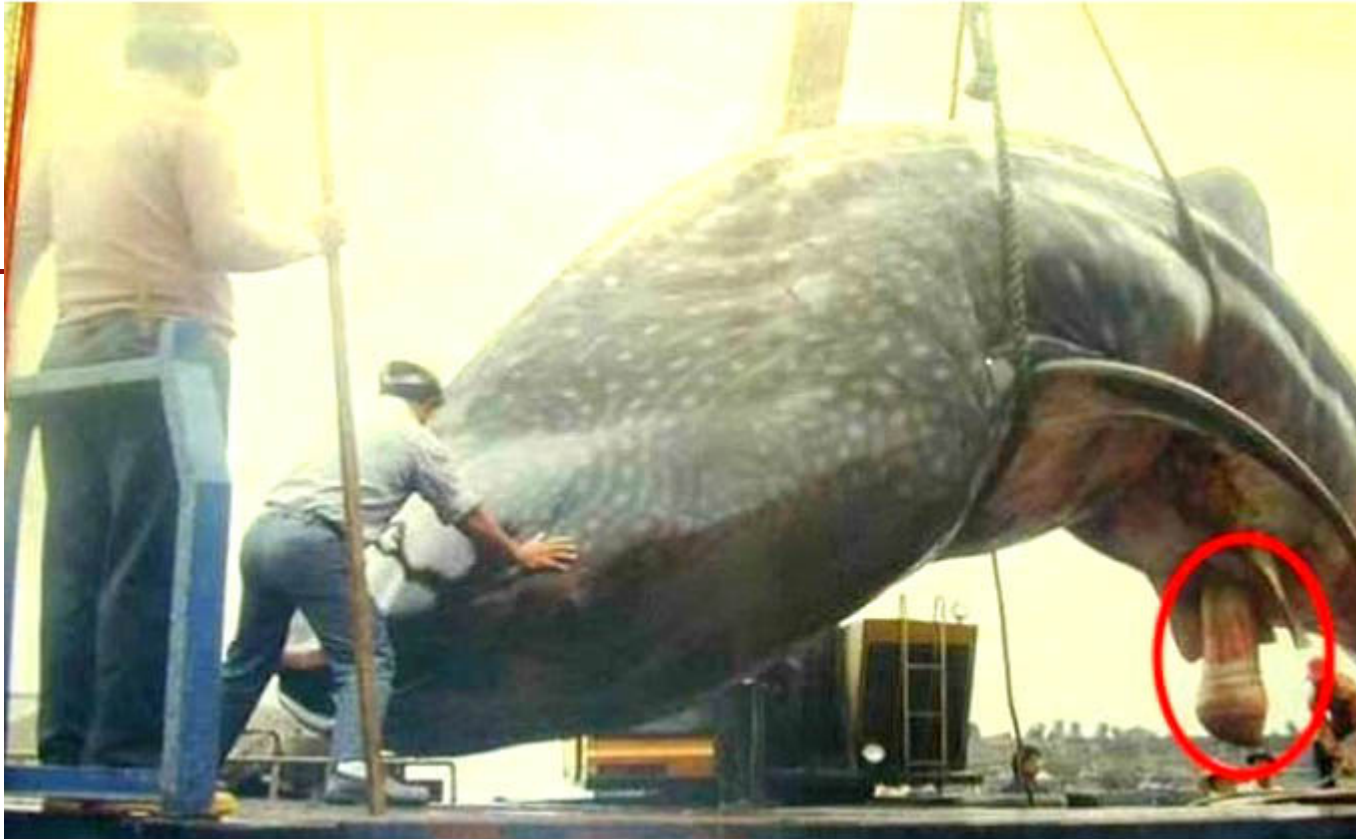
W5 Situated near the top of this hierarchical model is.....

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W26 The first strategy we used is based on the component of biological variation. To understand why the biological variation has to be taken into account, let's look at an example. When measuring the volume of a sample of human semen if we are off by 1ml this is a big mistake.

however if we are measuring the volume of blue whale semen, a mistake of 1ml, is just a salty drop in the ocean.

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The blue whale produces 1,600 liters of sperm each year when it ejaculates; but only deposits 10% of it, in its partner; that is to say that 1,400 liters remain scattered in the ocean.

So you ask why the water is so salty?

Don't drink the water!!

Slide 21

W12

But if you are measuring the volume of a blue whale, 1 ML of semen is just a salty drop in the ocean.

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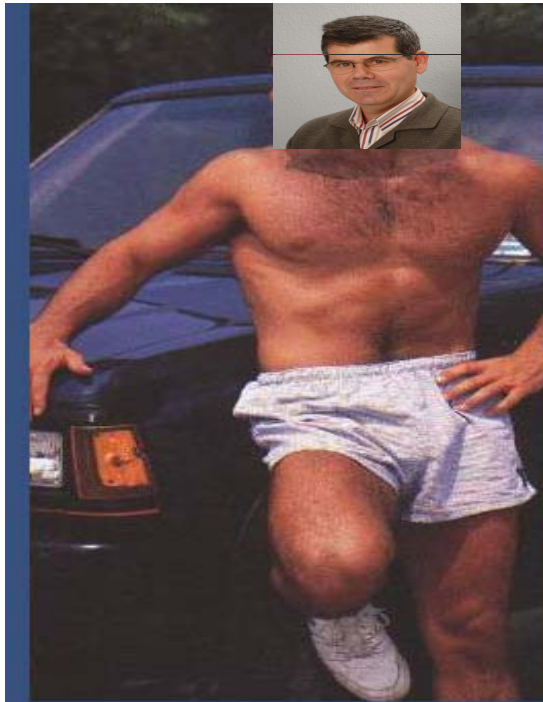
Biological variation of seminal parameters in healthy subjects

C.Álvarez^{1,4}, J. A.Castilla^{2,5}, L.Martínez², J. P.Ramírez³, F.Vergara³ and J. J.Gaforio⁴

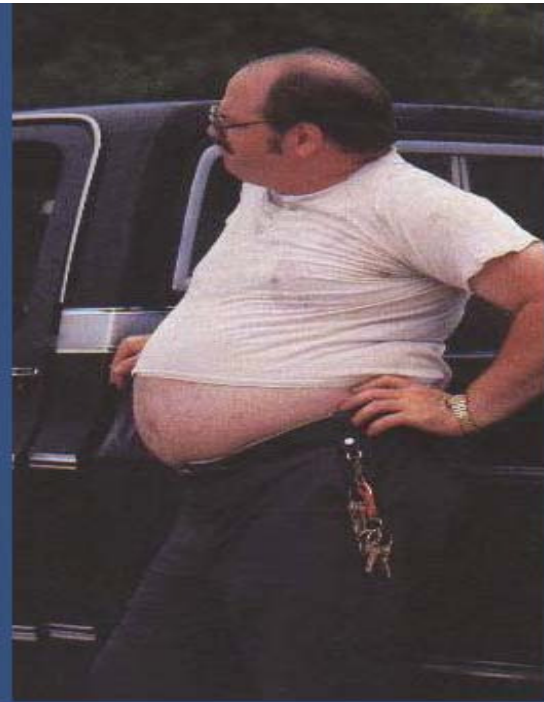
¹Unidad de Reproducción, Clínica Avicena, Jaén, ²Unidad de Reproducción, Hospital Virgen de las Nieves, Granada, ³CEIFER, Granada and ⁴Departamento de Ciencias de la Salud, Universidad de Jaén, Jaén, Spain

⁵To whom correspondence should be addressed at: Unidad de Reproducción, Hospital Virgen de las Nieves, E-18014, Granada,

What she wants...



What she has..



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Slide 22

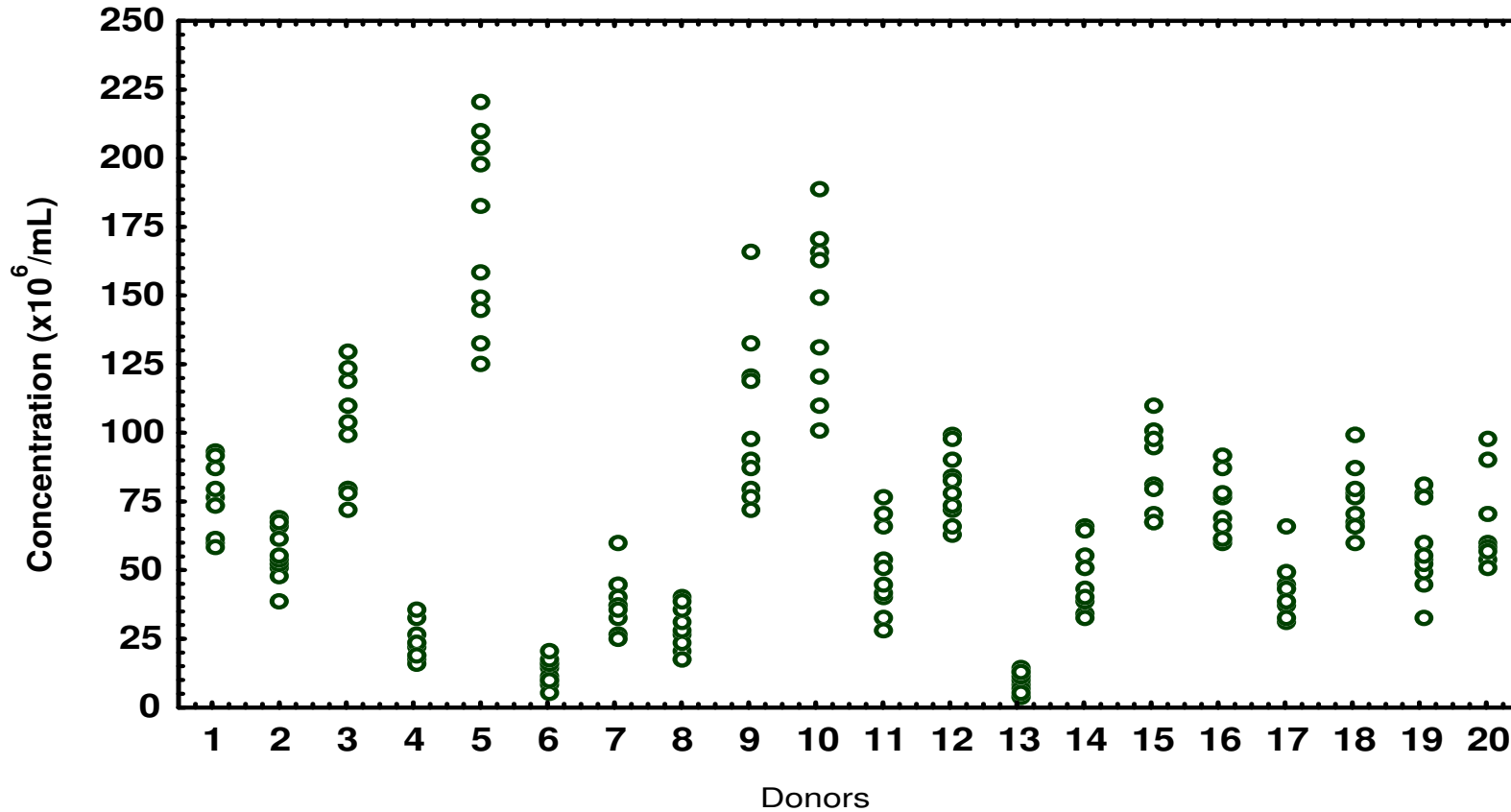
JL1

Jose Luis; 30/09/2009

Biological variation of seminal parameters in healthy subjects

C.Álvarez^{1,4}, J. A.Castilla^{2,5}, L.Martínez², J. P.Ramírez³, F.Vergara³ and J. J.Gaforio⁴

¹Unidad de Reproducción, Clínica Avicena, Jaén, ²Unidad de Reproducción, Hospital Virgen de las Nieves, Granada, ³CEIFER, Granada and ⁴Departamento de Ciencias de la Salud, Universidad de Jaén, Jaén, Spain



Hierarchy of models to set analytical quality specifications in laboratory medicine (Kenny et al., 1999).

1. Evaluation of the effect of analytical performance on clinical outcomes in specific clinical settings

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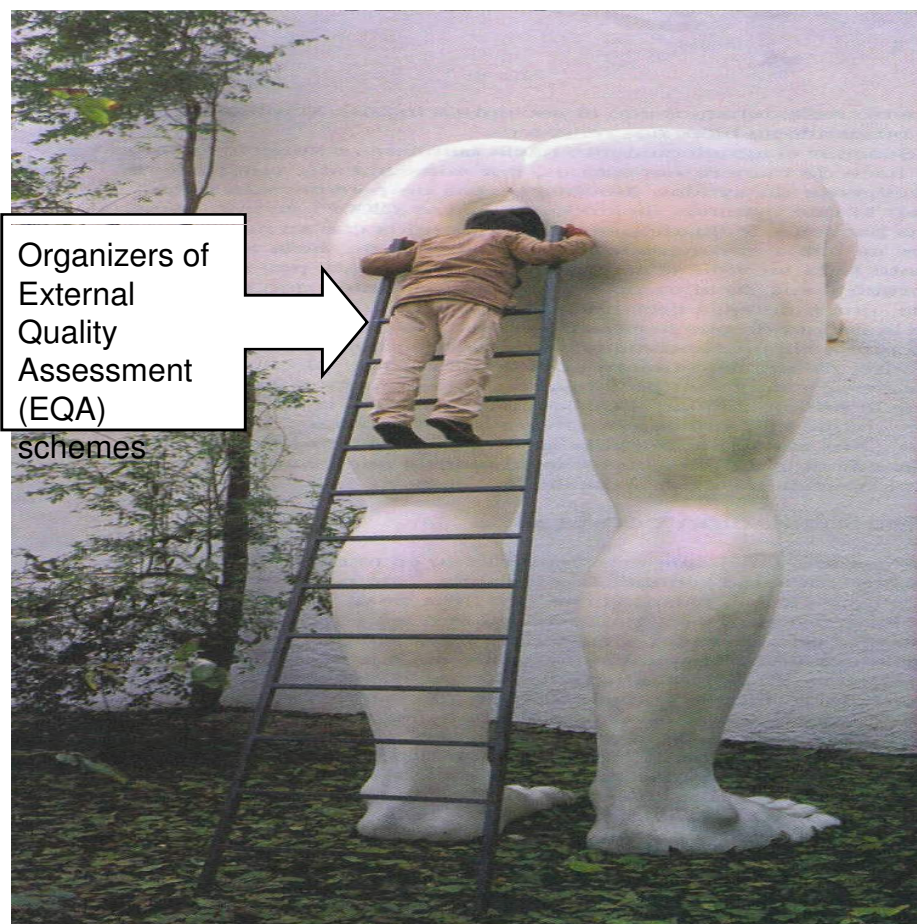
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b. As found in current publications on methodology.

W15 External quality control program for semen analysis: Spanish experience

C. Álvarez,^{1,4} J. A. Castilla,² J. P. Ramírez,³ F. Vergara,³ A. Yoldi,³ A. Fernández,³ and J. J. Gaforio¹



D. Cerny
Futura Art Gallery, Praga

Slide 25

W15

The Eqa has a undeserved reputation since many professionals think its only function is checking up on their work, which can make them feel uncomfortable.

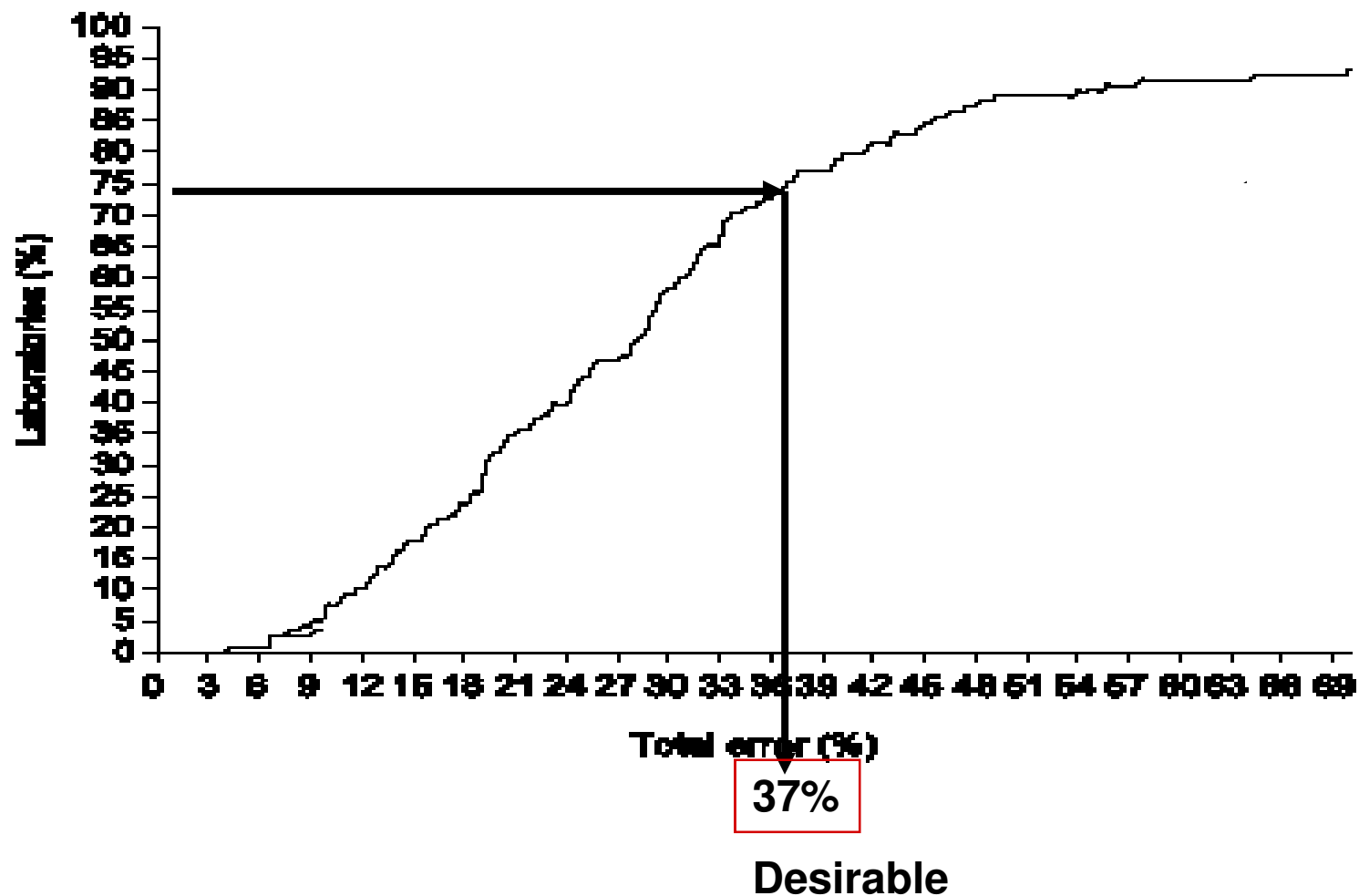
But in reality it can help them improve the quality of semen analysis, thus validating their results.

AlbayCF; 17/09/2009

W14 2005

Quality specifications for seminal parameters based on the state of the art

J.A.Castilla^{1,2,7}, J.Morancho-Zaragoza³, J.Aguilar^{2,4}, R.Prats-Gimenez³, M.C.Gonzalvo^{2,5}, E.Fernández-Pardo³, C.Álvarez^{2,6}, R.Calafell³ and L.Martinez¹



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W14

We consider that the quality specification should be the maximum percentage of total error committed by the top 75% of laboratories participating in the EQA.

Now in this graph obtained from sperm concentration results in the Spanish EQC, you can see that maximum percentage of total error committed by the top 75% of laboratories participating in the EQA was 37%.

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Hierarchy of models to set analytical quality specifications in laboratory medicine (Kenny et al., 1999).

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2007

ORIGINAL ARTICLE

Quality specifications for seminal parameters based on clinicians' opinions

J. AGUILAR¹, C. ÁLVAREZ², J. MORANCHO-ZARAGOZA³,
R. PRATS-GIMENEZ³, J. P. RAMÍREZ⁴, E. FERNÁNDEZ-PARDO³,
L. MARTÍNEZ⁵, R. CALAFELL³, I. DURAN⁶ & J. A. CASTILLA^{4,5}

¹Banco de Semen CEIFER, Granada, Spain, ²Departamento de Ciencias de la Salud, Universidad de

Case History No. 2

Male, 29 years old, with a left varicocele in bipedestation position, normal breathing (Valsalva free), evident to the touch but not visible. Semen analysis carried out following WHO (1999) recommendations (after 72 hours' sexual abstinence, 20 minutes from collection to delivery; complete sample)

Volume: 3 mL

Concentration: **20** ill/mL

Type "a" motility: 5%

Type "b" motility : 10%

Type "c" motility : 25%

Morphology : 4%

Vitality: 50%

3 months after the varicocelectomy, even if you would not have considered it necessary, the semen analysis was repeated.

By how much should the reported values vary for you to consider this intervention to have worsened seminal quality?

By how much should the reported values vary for you to consider this intervention to have improved seminal quality?

Slide 28

W8

We surveyed clinicians on the topic of seminal parameters, with the aim of finding out when they consider a significant change has occurred in the sperm concentration, for a patient treated for varicocele.

The level of precision required on the laboratory will depend on the amount of change determined by the survey results. The higher the change, the lower required precision.

If clinicians consider a man's results has changed significantly when his results increase from 20 mill/mL to 30 mill/mL (50%), the precision of the andrology laboratory is of high importance due to the necessary level of precision to measure the difference between samples. However if they consider that a man's results have changed when his results increase from 20 mill-ml to 60 mill-ml a 300% increase the precision is of low importance.

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5.- Quality specifications of analytical CV for seminal parameters according to different strategies

	Minimum			
	Biological variation (Álvarez et al, 2003)	State of the art (Castilla et al, 2005)	Clinicians' opinions (Aguilar et al, 2008)	Mean
Concentration	20	18	10	16
Total motility	14	11	11	12
Progressive motility	11	10	12	11
Type "a" progressive motility	14	24	22	20
Morphology	15	29	14	19
Vitality	8	12	7	10

Slide 29

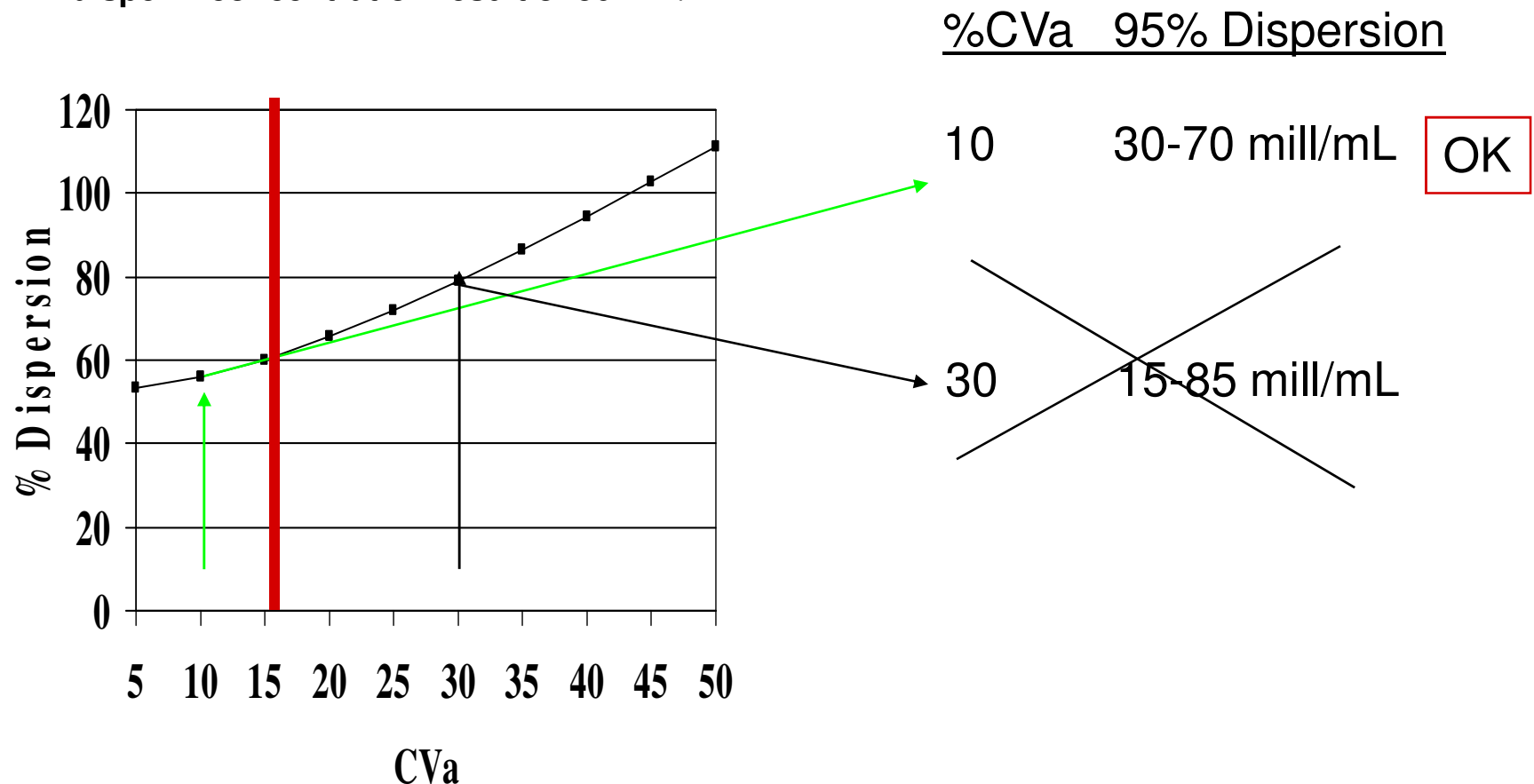
W27

please remember that the CV was 16

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Why is the analytical CV important?

Effect of imprecision on the 95% dispersion of a sperm concentration result of 50 mill/mL



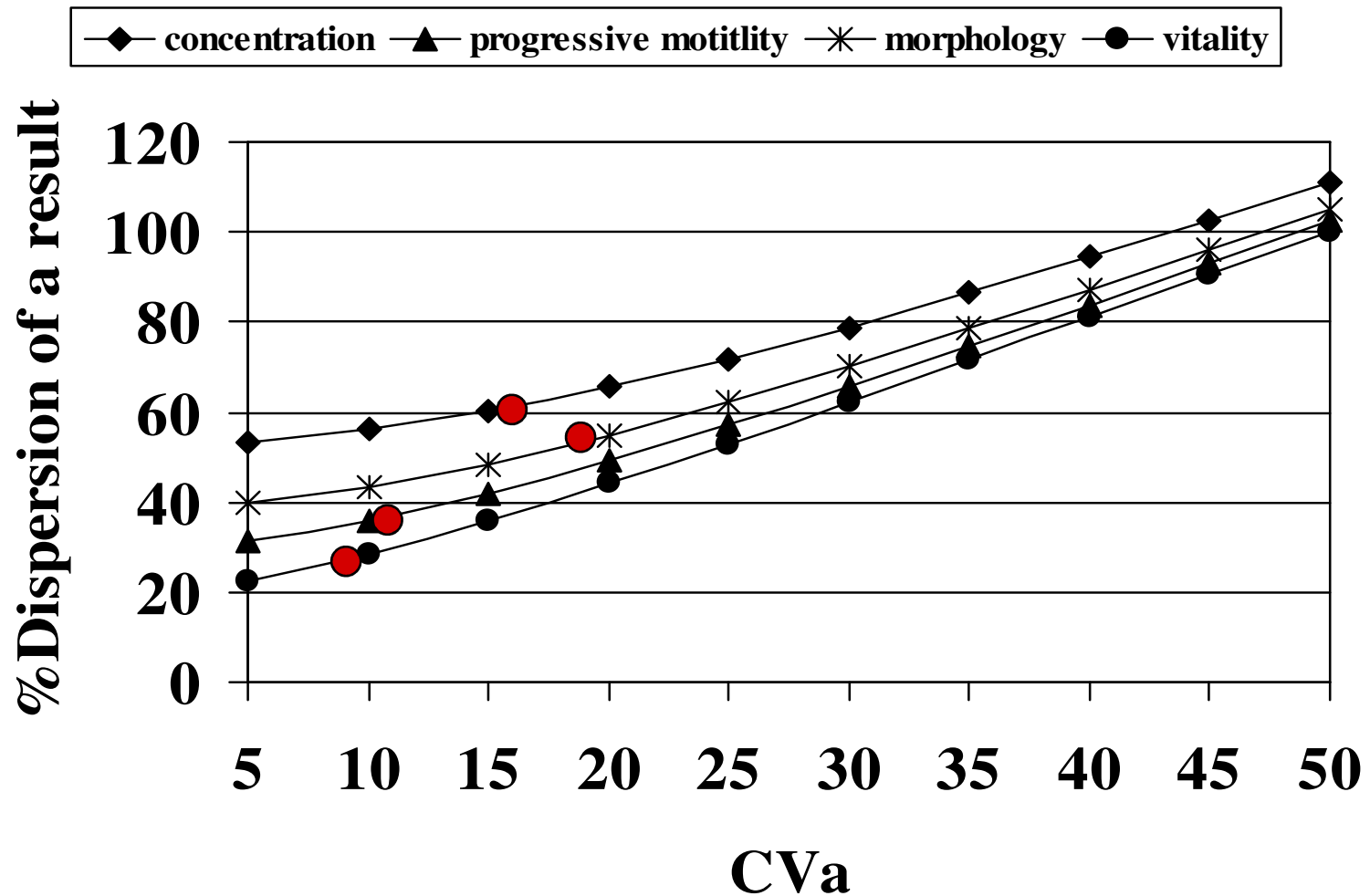
Castilla et al., Hum Reprod 2006;

Slide 30

W28

probably because the treatment the couple would undergo would depend on whether they recieved a result of 15mll-ml or 85 mill-ml.
AlbayCF; 15/09/2009

Why is the analytical CV important?



6.- What other uses are there for quality specifications?

- To design internal quality control programmes (Westgard et al., 2007)
- To evaluate in vitro diagnostic systems (Powers and Greenberg, 1999)
- To assess laboratory reliability performance in EQA (Sciacovelli et al., 2004)
- To share reference intervals (Ricos et al., 2004)

Slide 32

W29 What other uses are there for Quality specifications

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W30 we have to obligate the in vitro

what else can we do with qc?

AlbayCF; 17/09/2009

7.- Who should set the quality specifications for seminal parameters?

(Haugen, Castilla and Björndahl , 2009 unpublished)

- Laboratory andrology lacks a global professional organization
- The WHO manual is neither a permanent task force nor part of a global network
- Responsibility of the scientific organizations related to the investigation and treatment of male reproductive functions

Slide 33

W31 and last but not least.....
AlbayCF; 15/09/2009

W32 thus we consider that the responsibility.....
AlbayCF; 15/09/2009

7.- Who should set the quality specifications of seminal parameters?

(Haugen, Castilla and Björndahl , 2009 unpublished)

- **An international task force for clinical laboratory andrology**, supported by the above mentioned organization, which should
 - endorse the implementation of appropriate methods for laboratory analyses and quality control, including quality specifications
 - develop standardized training courses and stimulate the growth of a network of schemes for external quality control.
 - Furthermore, guidelines for journals publishing studies based on clinical laboratory andrology

Slide 34

W33

and finally, last but not least.....

AlbayCF; 17/09/2009

Conclusions

- **We need quality specifications (ISO: 15189, clinical use of semen analysis, etc...)**
- **We can set quality specification...**

BECAUSE WE ARE

Hierarchical model to set analytical quality specifications in laboratory medicine (Kenny et al., 1999).

1. Evaluation of the effect of analytical performance on clinical outcomes in specific clinical settings
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W9

Situated at the top of this hierarchtcal model is.....

AlbayCF; 20/07/2009

Thank you for your attention



Set

quality
specifications for
seminal parameters

W10

W34

I hope to return your hospitality, next March in my hometown



Special Interest
Group of Andrology

**Sperm and testicular
tissue banking**

ESHRE Campus 2010

Granada, Spain 25 & 26 March 2010

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W10

I hope to return your hospitality, when you visit my hometown next march to attend the Eshire campus in Granada Spain.

AlbayCF; 15/07/2009

W34

if you come, you will find out why we call it painful friday.

AlbayCF; 17/09/2009

Thank you for your attention



My children:

Pilar and Jose Antonio, who are also learning about quality control

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W35

If you come you will be able to visit the scale gate, with my children who will be pleased to answer all your questions about Granada.

AlbayCF; 17/09/2009

W37

W38

I hope to return your hospitality, next March in my hometown



Special Interest
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ESHRE Campus 2010

Granada, Spain 25 & 26 March 2010

Slide 40

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AlbayCF; 15/07/2009

W38 if you come, you will find out why we call it painful friday.
AlbayCF; 17/09/2009



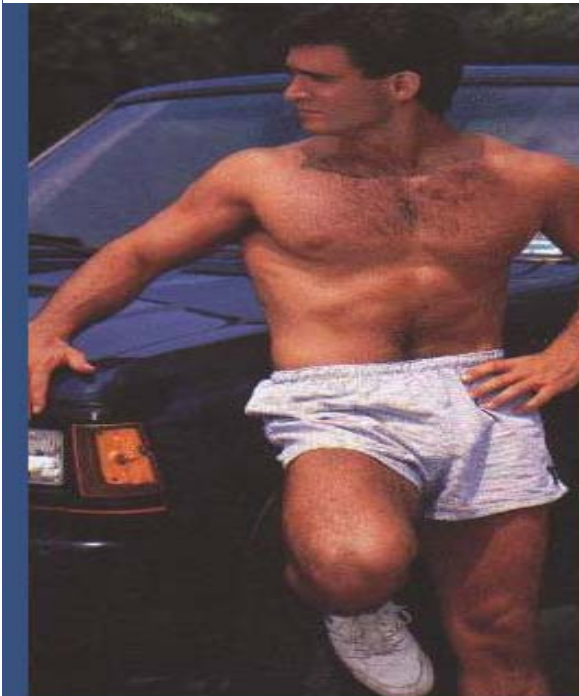
Biological variation of seminal parameters in healthy subjects

C.Álvarez^{1,4}, J. A.Castilla^{2,5}, L.Martínez², J. P.Ramírez³, F.Vergara³ and J. J.Gaforio⁴

¹Unidad de Reproducción, Clínica Avicena, Jaén, ²Unidad de Reproducción, Hospital Virgen de las Nieves, Granada, ³CEIFER, Granada and ⁴Departamento de Ciencias de la Salud, Universidad de Jaén, Jaén, Spain

⁵To whom correspondence should be addressed at: Unidad de Reproducción, Hospital Virgen de las Nieves, E-18014, Granada,

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What she has..

