Evaluarea incarcarii virale HIV-1, subtip F cu sistemul Cobas AmpliPrep-CobasTaqMan (CAP-CTM)

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Motivatia studiului

Infectia HIV beneficiaza de tratament etiologic (antiretroviral, **TARV**) care asigura **intarzierea progresiei clincie a bolii** si **profilaxia transmiterii materno-fetale si orizontale** a virusului;

Succesul clinic al TARV este dependent de **momentul initierii** si **controlul eficient al replicarii virale** prin TARV; in acest sens exista recomandari internationale care se bazeaza pe **evaluarea periodica a nivelului incarcarii virale HIV** (VL-HIV), alaturi de evaluarea clinica si cea imunologica

→→De corectitudinea evaluarii VL-HIV depinde succesul clinic al TARV

Premize virusologice

HIV este un virus cu variabilitate genetica deosebit de mare; distributie geografica: in tarile dezvoltate - subtip B, in tarile subdezvoltate – subtipuri non-B

Primele instrumente de evaluare a VL-HIV au fost bazate pe tehnica PCR (*end point*), si au fost construite pe modelul molecular al HIV subtip B

Tinta preferata pentru design-ul molecular al testelor pentru VL-HIV = **gena *gag***, foarte bine conservata

Premize clinico-epidemiologice

Infectia HIV, tratata sau chiar si netratata, poate evolua pe durata mai multor **zeci de ani**

HIV poate dezvolta **rezistenta la TARV → *switch* al schemei la un anume nivel VL-HIV**

Circulatia internationala a subtipurilor HIV determina aparitia de **variante recombinante**

Metode de evaluare a VL-HIV (1)

RT-PCR clasic (end point)/ NASBA/**rt RT-PCR**

Tehnologii de extractie manuala / **automatizata**

Non-IVD (*in house,* RUO) / **IVD**

Avantaje	**Dezavantaje**
Sensibilitate (sub 50 cp/mL)	costuri, contam. (?), ***false failure***
Linearitate mare (≥7 log)	costuri
Alte performante tehnologice	costuri, service
Calificare + timp personal lab.	costuri, service
Acceptarea internationala	**variabilitatea genetica**

Metode de evaluare a VL-HIV (2)

Discordante intre metodele IVD

AMPLICOR (CA) vs. NASBA

AIDS. 1997 Jun;11(7):859-65.

Subtype-specific problems with quantification of plasma HIV-1 RNA.

Alaeus A, Lidman K, Sönnerborg A, Albert J.

Division of Infectious Disease, Karolinska Institute, Danderyd Hospital, Sweden.

CONCLUSIONS: The HIV monitor assay and, possibly to slightly lesser degree, the NASBA assay appear unable to accurately quantify HIV-1 RNA levels in plasma samples from many subtype-A-infected individuals. These problems are likely to be due to primer mismatches and they limit the possibility of using these assays for routine monitoring of HIV-1-infected individuals in many parts of the world.

Concluzia autorilor: NASBA subevalueaza fata de CA pentru subtipul A

NOTA: CA = metoda standard pentru studiile clinice pentru timp indelungat

Nici un test pe subtip F in aceasta analiza

Metode de evaluare a VL-HIV (3)

Discordante intre metodele IVD

COBAS AMPLICOR (CA) vs. COBAS TAQMAN (CTM) v1

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2007, p. 3436–3438 Vol. 45, No. 10
0095-1137/07/$08.00+0 doi:10.1128/JCM.00973-07

Human Immunodeficiency Virus Type 1 (HIV-1) Plasma Load Discrepancies between the Roche COBAS AMPLICOR HIV-1 MONITOR Version 1.5 and the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Assays▽

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Received 10 May 2007/Returned for modification 11 July 2007/Accepted 27 July 2007

We compared plasma viral load values obtained with COBAS AMPLICOR human immunodeficiency virus type 1 (HIV-1) MONITOR version 1.5 and with COBAS TaqMan HIV-1 assays. Mean values were 4.2 and 2.9 $\log_{10}$ copies/ml, respectively, showing the lack of agreement between the two assays.

34 pacienti, B si non- B; un singur pacient cu subtip F, diferenta 1 log, detectabile

Duiculescu et al., Iasi 2008 – 9 cazuri Romania, diferenta 2.27±0.95 log cp/mL;
un caz (pacient naiv) cu 5.69 log cp/mL in CA si ND in CTM v1

Metode de evaluare a VL-HIV (4)

Discordante intre metodele IVD

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2008, p. 2918–2923 Vol. 46, No. 9
0095-1137/08/$08.00+0 doi:10.1128/JCM.02414-07

Performance of Three Commercial Viral Load Assays, Versant Human Immunodeficiency Virus Type 1 (HIV-1) RNA bDNA v3.0, Cobas AmpliPrep/Cobas TaqMan HIV-1, and NucliSens HIV-1 EasyQ v1.2, Testing HIV-1 Non-B Subtypes and Recombinant Variants▽

TABLE 3. Performance of three viral load assays testing 55 non-B specimens with detectable plasma viremia by at least one of three techniques[a]

Subtype at *pol** (no. of specimens)[b]	No. of specimens with:					
	≥0.5-log viremia differences			≥1-log viremia differences		
	V vs T	V vs N	T vs N	V vs T	V vs N	T vs N
A (2)	0	1	1	0	1	0
C (2)	0	0	0	0	0	0
D (1)	1	1	0	1	1	0
F2 (1)	0	0	0	0	0	0
G (11)	4	5	6	1	1	2
H (3)	0	0	0	0	0	0
CRF02_AG (23)	10	13	5	2	3	1
CRF10_CD (4)	0	3	3	0	0	0
CRF11_cpx (1)	0	1	1	0	0	0
URF (7)	0	1	0	0	0	0
Total (%)	15 (27.3)	25 (45.5)	16 (29)	4 (7.3)	6 (10.9)	3 (5.4)

[a] Abbreviations: V, Versant HIV-1 RNA 3.0; T, Amplicor Cobas TaqMan 48; N, NucliSens HIV-1 EasyQ v1.2.
[b] Only plasma specimens considered with >50 HIV RNA copies/ml.

Metode de evaluare a VL-HIV (5)

Discordante intre metodele IVD

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2010, p. 1195–1200 Vol. 48, No. 4
0095-1137/10/$12.00 doi:10.1128/JCM.01832-09

Performance Evaluation of the New Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 Test Version 2.0 for Quantification of Human Immunodeficiency

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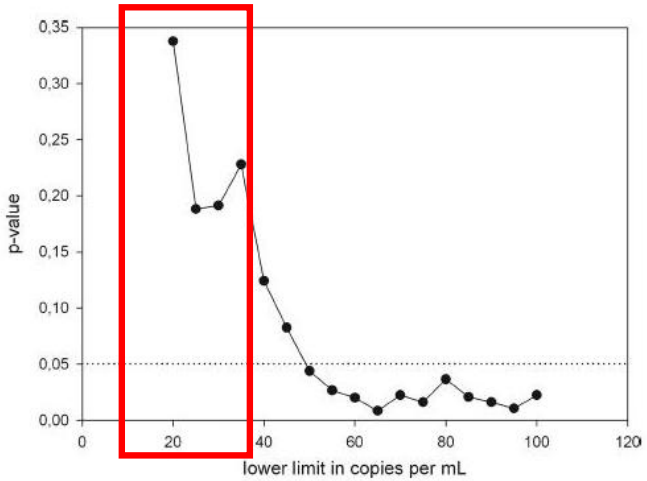

FIG. 3. Probability of treatment failure for patients showing levels below the threshold of quantification for CAP/CA v1.5 who were retested with CAP/CTM v2.0. A 5-cp/ml moving lower limit was set, and the probability of therapy failure for patients with levels below this lower limit was compared to that for patients with levels above this limit and was tested for significance. The figure shows the *P* values for each limit ($P < 0.05$ is significant).

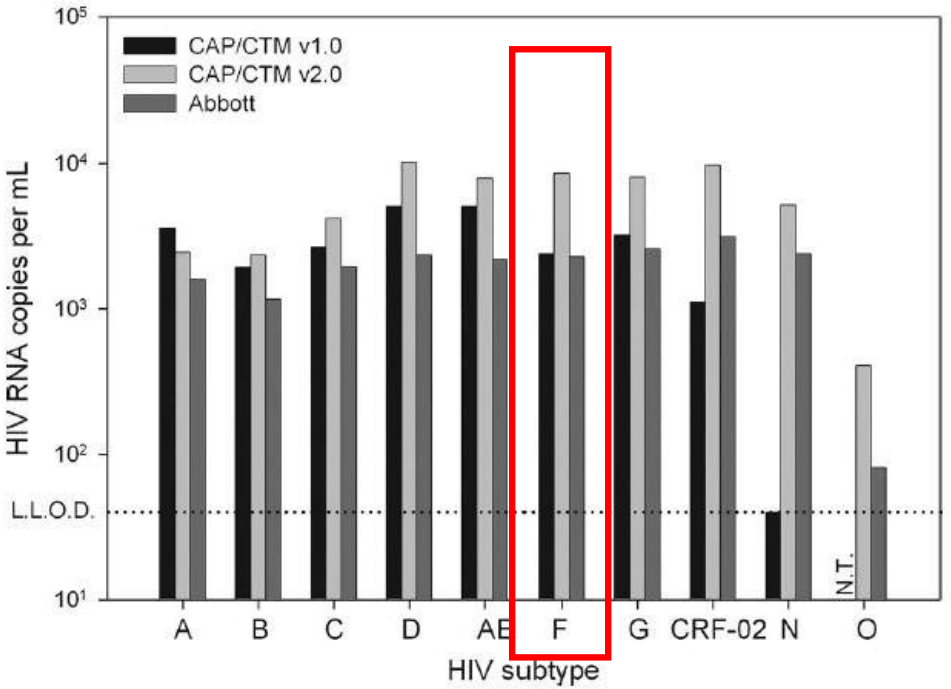

FIG. 5. Viral loads for the WHO 1st reference panel as determined by CAP/CTM v1.0, CAP/CTM v2.0, and the Abbott assay. N.T., not tested.

Metode de evaluare a VL-HIV (6)

Discordante intre metodele IVD

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2010, p. 1337–1342 Vol. 48, No. 4
0095-1137/10/$12.00 doi:10.1128/JCM.01226-09

Correction of Underquantification of Human Immunodeficiency Virus Type 1 Load with the Second Version of the Roche Cobas AmpliPrep/Cobas TaqMan Assay▽†

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Received 23 June 2009/Returned for modification 18 September 2009/Accepted 4 February 2010

Concluziile autorilor:

1. **CAP-CTM v1 subevalueaza VL-HIV cu ÷0.32 log fata de CA (10% ≥0.71 log)**
2. **8/19 (42%) din subevaluarile ≥0.93 log ale CAP-CTM v1 sunt la subtip B**
3. **CAP-CTM v2 nu subevalueaza comparativ cu CA (diferenta ÷ 0.08 log)**

Obiectivul studiului

Evaluarea CTM v1 vs. CAP-CTM v2
a plasmelor de la pacienti infectati cu
HIV-1 subtip F1

Pacienti, materiale si metode

Pacienti:

- **71** adulti infectati HIV-1, **86 plasme** (Spital Babes: 2005-2010)
- **18** dintre ei au test genotipic de rezistenta la TARV
- →subtipare fasta pe fragmentele de gena *pol* secventiate (REGA subtyping tool, www.hivdb.Stanford.edu si www.bioafrica.net); **genotip F1** (*nu am avut cazuri non-F1 disponibile*)

Evaluarea incarcarii virale:

- **Cobas TaqMan HIV-1 cu extractie manuala (HPS) = CTM v1**

LLOD = 47 cp/mL; linearitate → 10E7 cp/mL; **0.5 mL/test**

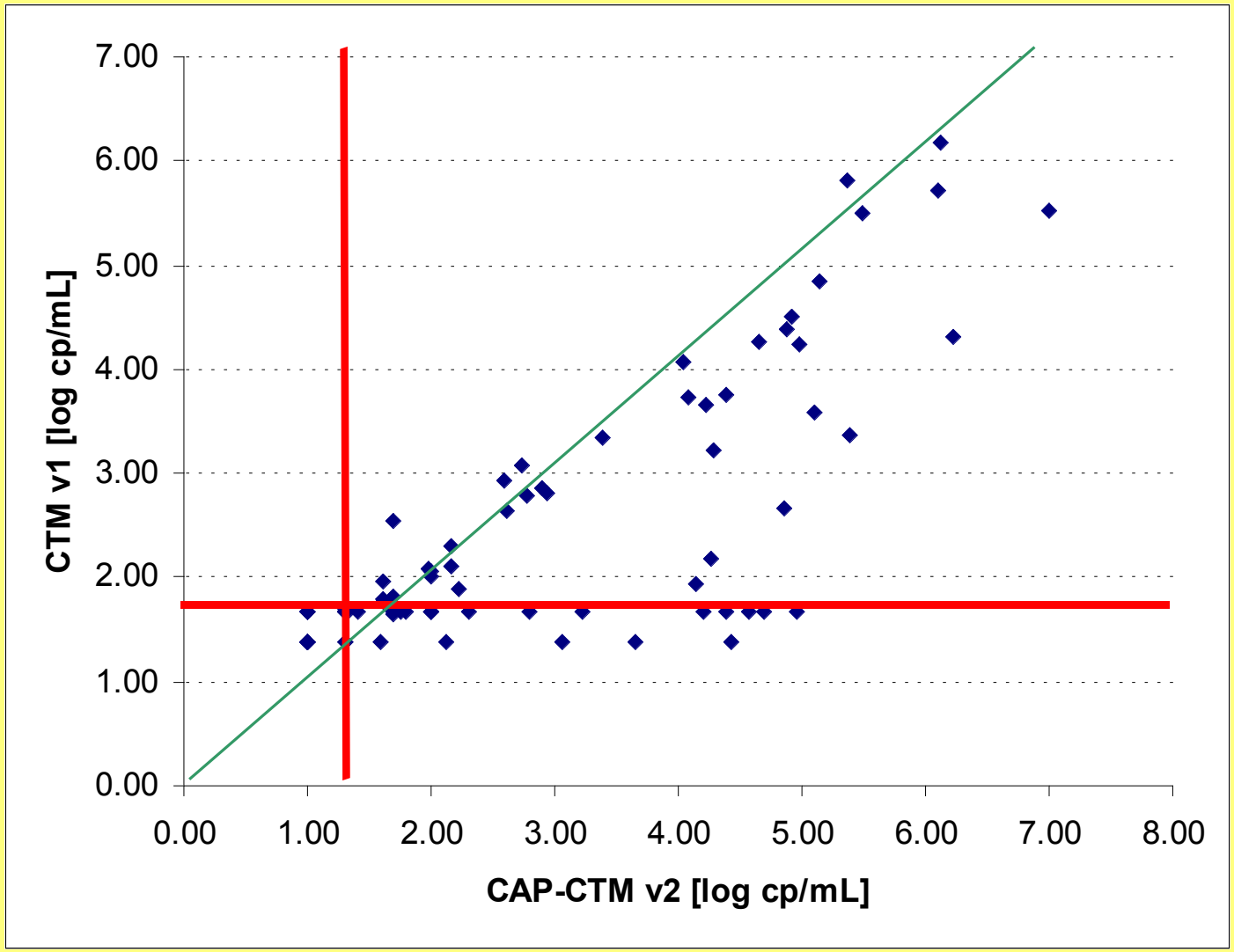
- **Cobas TaqMan HIV-1 v2 cu extractie automata = CAP-CTM v2**

LLOD = 20 cp/mL; linearitate → 10E7 cp/mL; **1 mL/test**

design molecular nou al testului, cu doua perechi de primeri si sonde

Rezultate (1)

86 testari CTM v1 vs. CAP-CTM v2



Conventie:

CTM v1

ND = **1.37**

Detectabil <47 = **1.67**

CAP-CTM v2

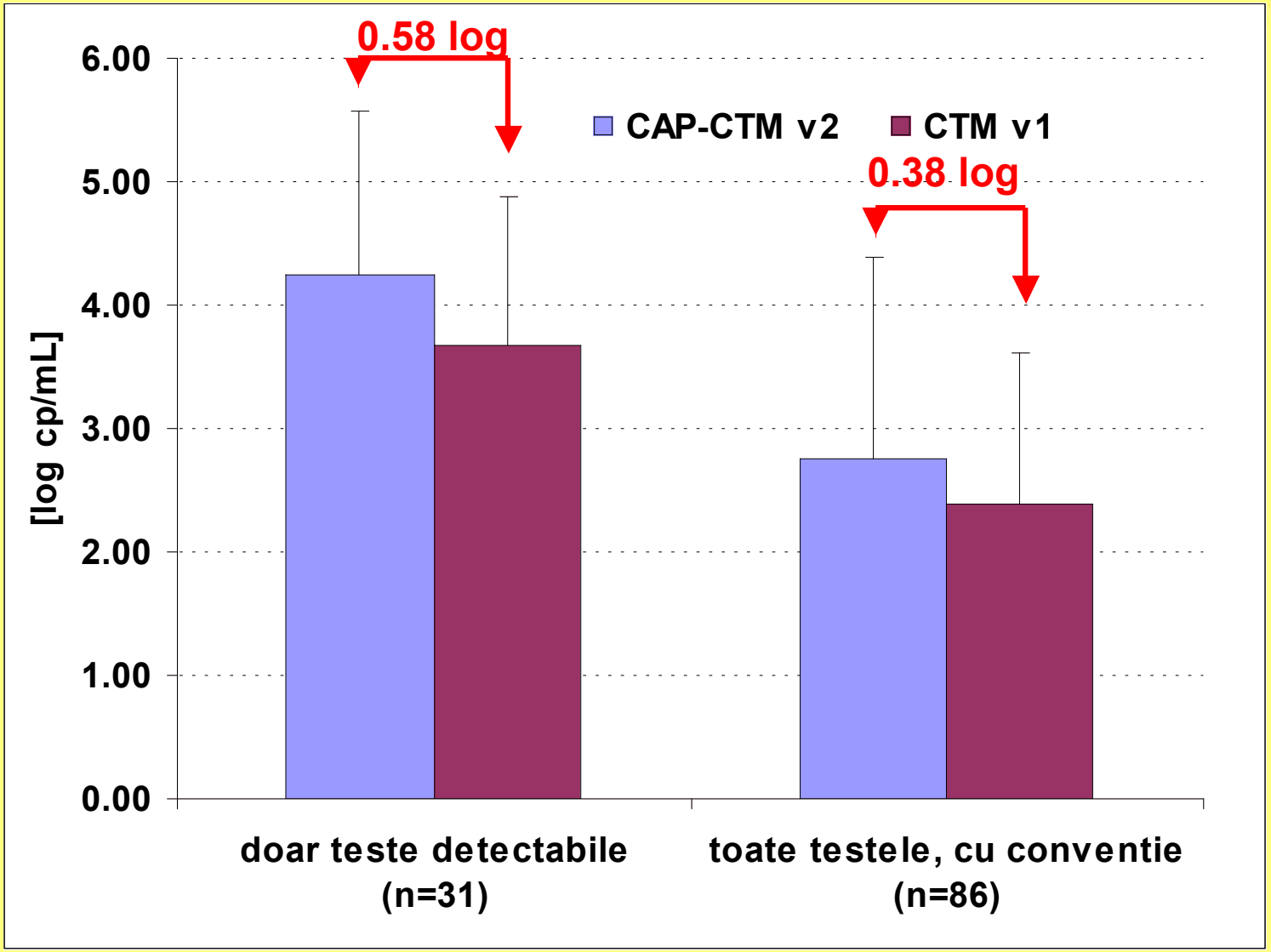
ND = **1.00**

Detectabil <20 = **1.30**

[log cp/mL]

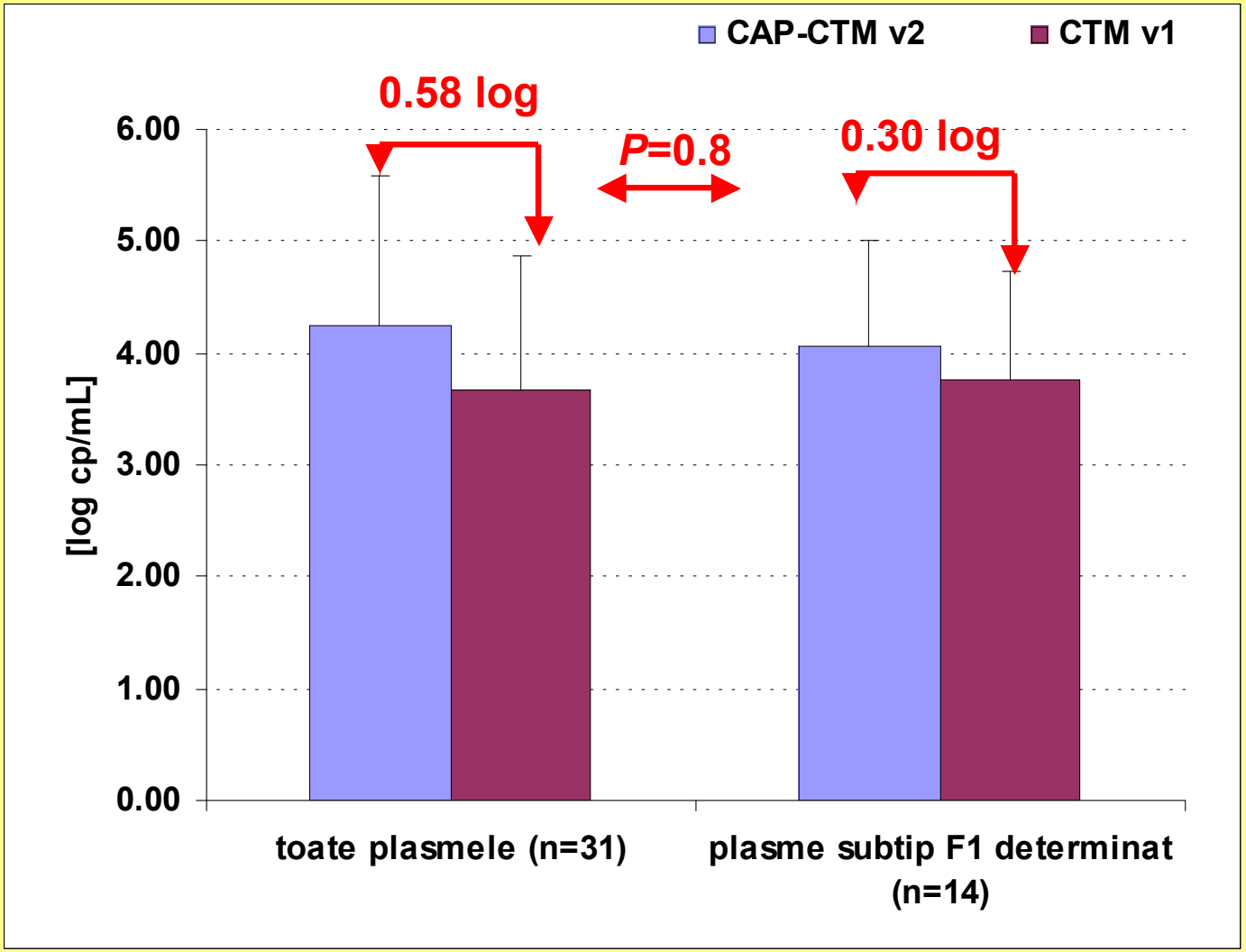
Rezultate (2)

86 testari CTM v1 vs. CAP-CTM v2



Rezultate (3)

subtip F1 in selectia de plasme detectabile



Rezultate (4)

discordante CAP-CTM v2 – CTM v1

23 din 86 **(26,7%)** testari comparative (selectie!) au avut o diferenta > 0,5 log cp/mL

18 **(20,9%)** au avut **diferenta > 1,0 log cp/mL**.

In 7 cazuri valoarea diferentei a fost negativa dar < 0,5 log cp/mL ca valoare absoluta

Concluzii

CAP-CTM v2 produce valori similare **sau mai mari** comparativ cu CTM v1 pentru evaluarea VL-HIV subtip F1 (~*CTM v1 subevalueaza in cel putin ¼ cazuri*).

Un total de 15 (**17%**) din cazurile comparate (!) au avut o subevaluare a VL-HIV prin CTM v1 fata de CAP-CTM v2 cu **potential de a influenta decizia clinca**.

Subevaluarile CTM v1 fata de CAP-CTM v2 pot fi puse **pe seama design-lui molecular al testului** si/sau a erorilor de operare in procedura de extractie manuala a ARN.

Multumiri

Clinica Spital Babes:
Dr. Cristiana Oprea, Dr. Roxana Radoi, Dr. Luminita Ene, Dr. Ina Ungureanu, Dr. Cristina Patru, Dr. Silvia Verescu, Dr. Simona Erscoiu, Dr. Dan Duiculescu, Prof. Dr. Emanoil Ceausu

Laborator Diagnostic Molecular INBI Matei Bals:
Dr. Simona Paraschiv, As. Mihaela Tinischi, Dr. Dan Otelea

Laborator VIBM Spital Babes:
As. Mariana Barbu, As. Rodica Sarman, As. Malvina Stanciu

Roche Romania SRL, Divizia de Diagnostic
Dr. Doinita Ispas, Dr. Andreea Zaharia, Vladimir Celmare