PSA MAGLUMI
The ultrasensitive PSA assay has been recently acknowledged as a useful tool for the monitoring of patients prostatectomized for prostatic cancer. We have evaluated a commercially available ultrasensitive PSA assay (Immulite Third Generation PSA, DPC Los Angeles CA) in comparison with the routinely used PSA (Immulite PSA, DPC). When evaluated with different approaches, the analytical sensitivity of ultrasensitive PSA ranged between 0.0029 and 0.0038 ng/ml. The biological detection limit was 0.0098 ng/ml. Dilution of samples with low PSA levels showed a good recovery (from 88 to 113%) up to 1:128 dilution factor (final PSA levels ranging from 0.004 to 0.016 ng/ml in different samples). The assay precision was excellent in the low dose range, the highest interassay interadjustment CV among replicates being 5.84% when assaying serum samples with PSA lower than 1.0 ng/ml. Besides its role in the follow-up of prostatectomized patients, the evaluated ultrasensitive PSA could be reliably used for the detection of clinically meaningful PSA variations in the low dose range, and it could therefore be a candidate for the assessment of PSA velocity.

*Witherspoon LR, Lapeyrolerie T.*

... We measured PSA in 1,037 serum samples obtained serially from 127 men after radical prostatectomy using the IMMULITE Third Generation PSA assay. ...

The IMMULITE Third Gen. PSA assay has an analytical sensitivity of less than 0.002 ng/ml and a clinically useful decision threshold of 0.01 ng/ml ....

... The IMMULITE Third Gen. PSA assay provides clinically useful information not previously available from PSA assays with conventional sensitivity, which is highly predictive of cancer activity in patients within 2 years after radical prostatectomy.
Early detection of cancer relapse after prostatectomy using very sensitive prostate-specific antigen measurements.

Witherspoon LR.

... The improved clinical detection limit provided by the Immulite 3rd Gen assay (0.01 ng/mL) provided clinically useful information not previously available using assays of conventional sensitivity (Fig.4). ... All of the patients destined after prostatectomy to have biochemical and clinical cancer recurrence were correctly identified using the Immulite 3rd Gen assay by about 2 years after surgery. Conversely, patients at minimal or no risk of early cancer recurrence were also correctly identified. ...
Test PSA 3° Generazione vs test PSA convenzionali

Identificazione con IMMULITE

Identificazione con test Convenzionale

Anni di Opportunità

Ricorrenza del Cancro

PSA, ng/mL

0,20

0,10

0,01

Trattamento

Anni dopo trattamento del Cancro prostatico

10
Undetectable ultrasensitive PSA after radical prostatectomy for prostate cancer predicts relapse-free survival.

...Serum PSA levels were measured with the Roche COBAS CORE assay in the first 80 patients (lower detecting limit = 0.1 ng ml⁻¹). From 1997 onwards, 120 patients had measurements using the IMMULITE ‘Third Generation’ sPSA assay (Diagnostic Products Corporation, DPC, Gwynedd, UK). This assay detects PSA down to below levels of 0.01 ng ml⁻¹. Interassay variation is negligible at ≥ 0.01 ng ml⁻¹. This low detection level is a consequence of the efficient centrifugal wash, which results in a low nonspecific signal accompanied by a large specific signal afforded by the chemiluminescent label (Babson et al, 1991).

...CONCLUSIONS. The role of RRP has been controversial in the UK partly because of the scepticism regarding the effectiveness of the procedure. Undoubtedly, not all patients undergoing RRP are cured of prostate cancer and patient selection is an important factor. Patients are keen to know if they have been cured of cancer after RRP. Until recently, to predict this we have been reliant upon histology alone. However, ultrasensitive PSA assays now enable us more accurately to advise patients of their chance of PSA relapse within 2 months of surgery and appears to be a reliable predictor of cure of prostate cancer.
BMC Urol. 2014 Oct 2;14:79.
The use of early postoperative prostate-specific antigen to stratify risk in patients with positive surgical margins after radical prostatectomy. Vesely S1, Jarolim L, Duskova K, Schmidt M, Dusek P, Babjuk M.

...All the PSA tests were performed in a single hospital laboratory under standardized settings using the **Immulite third-generation PSA assay** (Diagnostic Products Corp, Los Angeles, California; lower detection limit 0.003 ng/ml). Biochemical recurrence was defined as a single post-nadir PSA level of 0.2 ng/ml or greater.

...Conclusions: The level of ultrasensitive PSA yields valuable information about the prostatectomy outcome already at the first month after the surgery and should aid risk stratification in patients with PSM. Patients not likely to experience subsequent disease progression may be spared the toxicity of immediate adjuvant radiotherapy.
La concentrazione di PSA in un dato campione determinata con dosaggi di diversi produttori può variare a causa delle differenze nei metodi utilizzati e nella specificità del reagente. I risultati comunicati dal laboratorio al medico devono includere le caratteristiche del dosaggio utilizzato. I valori ottenuti con diversi dosaggi del PSA non possono essere interscambiati. Prima di passare da un dosaggio all'altro, il laboratorio deve confermare i valori di base per i pazienti monitorati in serie.
Definizione di Generazioni dei test PSA totale

<table>
<thead>
<tr>
<th>Limite di Sensibilità</th>
<th>Generazione test PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Funzionale (anche detta: LoQ, Limit of Quantitation)</td>
<td>1 – 3 ng/mL</td>
</tr>
<tr>
<td>con CV inter-assay &lt;20%</td>
<td>0,1 – 0,3 ng/mL</td>
</tr>
<tr>
<td></td>
<td>0,01 – 0,03 ng/mL</td>
</tr>
</tbody>
</table>
How sensitive is a prostate-specific antigen measurement? How sensitive does it need to be?

Bock JL, Klee GG.

... many of the currently used assays have intralaboratory coefficients of variation greater than 20% for PSA concentrations less than 0.4 ng/mL. Also, there are major differences in the level of PSA reported by various assays in these low concentration samples. These level differences (if they are also seen in clinical samples) may cause clinical problems when fixed serum PSA thresholds (eg, 0.2 ng/mL) are used to make clinical decisions related to prostate tumor recurrence.
Autunno 2013: Dismissione PSA 3° Generazione IMMULITE 2000

SIEMENS

A91DX-CAI-130655-XC1-4A00

Importante Comunicazione relativa a prodotti
Annuncio di dismissione dal commercio dei kit
PSA di terza generazione per i sistemi IMMULITE 2000/2000 XPi

Soluzione:
PSA Maglumi !!!
Sensibilità Funzionale PSA Maglumi

Dati in File. Snibe, Schenzhen, Cina.
Misura di 5 campioni a basso livello di PSA (20 giorni consecutivi; 1 Lotto kit; 100 risultati)

Functional Sensitivity (Limit of Quantitation, LoQ, with a maximum CVt of 20%)

LoQ = 0,028

Diagramma con grafico di dispersione per la determinazione di Functional Sensitivity (Limit of Quantitation, LoQ) di PSA con un massimo CVt del 20%.

Reix N, Massart C, Gasser F, Heurtault B, Agin A.

DESIGN AND METHODS:
The functional sensitivity (FS) of the ADVIA Centaur system TSH assay was assessed. We report the values yielded for QC materials in two clinical laboratories.

RESULTS:
The FS was <0.02 mIU/L. The low-TSH QC (a serum pool) showed unacceptable between-lot imprecision (mean 0.0252 mIU/L, CV 22%).

CONCLUSION:
We do encourage healthcare laboratories to constitute low-TSH serum pools to ensure that the results they report meet 3rd-generation criteria.
Validazione PSA Maglumi

ELAS 2013

VALUTAZIONE DELLE PRESTAZIONI ANALITICHE DI MAGLUMI 2000 PLUS PER LA MISURA DELL’ANTIGENE PROSTATICO SPECIFICO (PSA)

Laboratorio Generale, Dipartimento Diagnostica di Laboratorio, Azienda Ospedaliero Universitaria Careggi, Firenze
Lo studio di imprecisione effettuato secondo il protocollo CLSI EP17-A2 su cinque livelli di concentrazione (medie: livello 1 = 0.017 ng/mL, livello 2 = 0.028 ng/mL, livello 3 = 0.037 ng/mL, livello 4 = 0.283 ng/mL, livello 5 = 0.879 ng/mL) in sei giorni e su due diversi lotti in parallelo, ha evidenziato un CV intra-assay, inferiore al 17% per il livello 1, inferiore al 13% per il livello 2, inferiore al 12% per il livello 3, inferiore al 10% per il livello 4, inferiore al 5% per il livello 5 e un inter-assay cumulativo dei due lotti inferiore a 18% per il livello 1 e il livello 2, inferiore al 12% per il livello 3, inferiore all'8% per il livello 4 e inferiore al 4% per il livello 5 (Tabella 1).

<table>
<thead>
<tr>
<th>Livello</th>
<th>CV intra-assay</th>
<th>CV inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (media 0.017 ng/mL)</td>
<td>&lt; 17 %</td>
<td>&lt; 18 %</td>
</tr>
<tr>
<td>2 (media 0.028 ng/mL)</td>
<td>&lt; 13 %</td>
<td>&lt; 18 %</td>
</tr>
<tr>
<td>3 (media 0.037 ng/mL)</td>
<td>&lt; 12 %</td>
<td>&lt; 12 %</td>
</tr>
<tr>
<td>4 (media 0.238 ng/mL)</td>
<td>&lt; 10 %</td>
<td>&lt; 8 %</td>
</tr>
<tr>
<td>5 (media 0.879 ng/mL)</td>
<td>&lt; 5 %</td>
<td>&lt; 4 %</td>
</tr>
</tbody>
</table>
Sensibilità Funzionale PSA Maglumi: verifica su 2 lotti kit

Dati in File. Medical Systems S.p.A.
Misura di 5 Pool di sieri a basso livello di PSA (Pool in triplo; 2 Lotti kit; 5 + 6 run; 165 risultati)

Functional Sensitivity (Limit of Quantitation, LoQ, with a maximum CVt of 20%)

LoQ = 0,019
Sensibilità Funzionale PSA Maglumi: verifica su 3 lotti kit


Misura di n.10 Pool a basso livello di PSA (5 Pool per 20 giorni con 1 Lotto kit, 100 risultati; 5 Pool in triplo; 2 Lotti kit; 5 + 6 run; 165 risultati)

Functional Sensitivity (Limit of Quantitation, LoQ, with a maximum CVt of 20%)

LoQ = 0,023
Profilo di Precisione PSA Maglumi: verifica su 3 lotti kit
(range campioni dosati: 0,010 – 123 ng/mL)


Profilo di 343 risultati complessivi, ottenuti da:
Misura di 10 Pool a basso livello di PSA:
5 Pool per 20 giorni con 1 Lotto kit (100 risultati) e
5 Pool in triplo; 2 Lotti kit; 5 + 6 run (165 risultati);
Misura di 4 Sieri individuali, in triplo su campioni
indiluiti e dopo diluizioni scalari (78 risultati)

\( \text{LoQ} = 0,023 \)

Functional Sensitivity
(Limit of Quantitation, LoQ, with a maximum CVt of 20%)
... Dilution of samples with low PSA levels showed a good recovery (from 88 to 113%) up to 1:128 dilution factor (final PSA levels ranging from 0.004 to 0.016 ng/ml in different samples).
Diluizione PSA Maglumi (siero tal quale = 123 ng/mL)
Dati in File, Medical Systems S.p.A.

Linearity

\[ y = 0.9879x + 4.1407 \]

\[ R^2 = 0.9856 \]
Diluizione PSA Maglumi (siero tal quale = 14,18 ng/mL)
Dati in File, Medical Systems S.p.A.

**Linearity**

\[ y = 0,9947x + 0,2285 \]

\[ R^2 = 0,9968 \]
Diluizione PSA Maglumi (siero tal quale = 4,04 ng/mL)
Dati in File, Medical Systems S.p.A.

Linearity

\[ y = 1.0237x + 0.0723 \]

\[ R^2 = 0.9993 \]
Diluizione PSA Maglumi (siero tal quale = 1,47 ng/mL)
Dati in File, Medical Systems S.p.A.

\[
y = 1,0222x + 0,0681 \\
R^2 = 0,9942
\]
Mione R, Barichello M, Sartorello P, Leon A, Barioli P, Gion M

Dilution of samples with low PSA levels showed a good recovery (from 88 to 113%) up to 1:128 dilution factor (final PSA levels ranging from 0.004 to 0.016 ng/ml in different samples).

Fig. 1 - Evaluation of accuracy using both routine and ultrasensitive PSA methods. Final PSA values were obtained by multiplying the PSA dose by each dilution factor.
Toward metrological traceability in the determination of prostate-specific antigen (PSA): calibrating Beckman Coulter Hybritech Access PSA assays to WHO standards compared with the traditional Hybritech standards

Conclusions: The WHO calibration yields results approximately 25% lower for fT PSA and fPSA values when compared with the conventional Hybritech calibration. Using the WHO-aligned PSA assay, a fT PSA cut-off of 3 μg/L should be considered in clinical practice, while %fPSA cut-offs could be retained.
Warning

PSA concentrations are dependent on the standard used to calibrate the assay. PSA concentrations based on calibration to the WHO 96/670 Reference Preparation will differ significantly from PSA concentrations based on calibration to the original Hybritech Tandem™-R assay. The concentrations are not interchangeable. If the calibration is changed, accepted laboratory practice is to establish a new baseline for patient monitoring.¹
Calibrazione PSA Access:
Hybritech non intercambiabile con WHO

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>R1</th>
<th>Access Hybritech PSA Reagent Packs</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Materials Required But Not Provided</th>
<th>1. Access Hybritech PSA Calibrators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cat. No. 37205</td>
</tr>
<tr>
<td></td>
<td>Two options for calibration are provided with the Access Hybritech PSA Calibrators, Hybritech calibration or WHO calibration.</td>
</tr>
</tbody>
</table>

- **Hybritech calibration**: concentrations are zero and approximately 0.5, 2.0, 10, 75 and 150 ng/mL
- **WHO calibration**: concentrations are zero and approximately 0.4, 1.7, 8, 58 and 121 ng/mL

**Limitations of the Procedure**

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.008–150 ng/mL Hybritech calibration or 0.008 – 121 ng/mL WHO calibration).
Analytical Sensitivity: 0.003 ng/mL

Functional Sensitivity: 0.01 ng/mL, as demonstrated by the studies summarized in the Precision section. (Functional sensitivity is defined as the lowest concentration that can be measured with an interassay CV of 20%).
Analytical Sensitivity: 0.04 ng/mL

Functional Sensitivity: (Functional sensitivity is defined as the lowest concentration that can be measured with an interassay CV of 20%).
Functional sensitivity: 0.03 ng/ml

The functional sensitivity is the tPSA concentration that can be reproducibly measured with an interassay coefficient of variation of $\leq 20\%$. 
Sensitivity
Functional

Functional sensitivity is defined as the lowest concentration that can be measured with an inter-assay coefficient of variation (CV) less than or equal to 20%. The calculated %CV for one reagent lot from all sites was plotted against the mean concentration of each panel. A parametric curve was fitted through the data, and the functional sensitivity was determined to be less than 0.05 ng/mL, which corresponded to less than 20% CV on the fitted curve.
Attention: This IFU complies with IVD directive 98/79/EC and is intended for use by customers operating in a member state of the European Union

ST AIA-PACK PSAII

INTENDED USE

ST AIA-PACK PSAII is designed for in vitro diagnostic use only for the quantitative measurement of prostate specific antigen (PSA) in human serum or heparinised plasma on Tosoh AIA System Analysers.

SENSITIVITY

The minimum detectable concentration (MDC) of prostate specific antigen is estimated to be 0.01 ng/ml. The MDC is defined as the concentration of PSA that corresponds to the rate of fluorescence which is two standard deviations from the mean rate of fluorescence of 5 repeat determinations by ST AIA-PACK PSAII CALIBRATOR (1).
The analytical sensitivity of the Tosoh PSA assay is 0.02 ng/mL, which was determined by running the 0 calibrator 20 times in the ultra-sensitive format and represents the addition of 2 standard deviations above the mean. The inter-assay coefficient of variation is 26.3% at a PSA of 0.11 ng/mL.

Sensibilità Funzionale: ~ 0.14 ng/mL

(lowest concentration that can be measured with an inter-assay CV of 20%)
15.10. Analytical and functional sensitivity

Analytical sensitivity:
Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 2 standard deviations.

Functional sensitivity:
The functional sensitivity (defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%)

<table>
<thead>
<tr>
<th></th>
<th>Analytical sensitivity</th>
<th>Functional sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIAISON® Analyzer family</td>
<td>0.09 ng/mL</td>
<td>0.15 ng/mL</td>
</tr>
</tbody>
</table>
Sensitivity

The functional assay sensitivity (20 % CV) has been assessed as being 0.16 ng/mL.
1) Traceability
To perform an accurate calibration, we have provided the test calibrators standardized against the WHO 1st Reference Reagent 96/670.

3) Functional Sensitivity
The functional sensitivity is defined as the concentration of total PSA at a total %CV exceeds 20%. The functional sensitivity of total PSA is less than 0.03ng/ml.
Sensibilità Funzionale (S.F.) e Range di Misura dei test PSA (ng/mL)

<table>
<thead>
<tr>
<th>Test</th>
<th>S.F.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA 3G Immulite 2000</td>
<td>0,01</td>
<td>20</td>
</tr>
<tr>
<td>PSA Immulite 2000</td>
<td>~0,20</td>
<td>150</td>
</tr>
<tr>
<td>PSA Roche Cobas</td>
<td>0,03</td>
<td>100</td>
</tr>
<tr>
<td>PSA Snibe Maglumi</td>
<td>0,03</td>
<td>400</td>
</tr>
<tr>
<td>PSA Abbott Architect</td>
<td>0,05</td>
<td>100</td>
</tr>
<tr>
<td>PSA DiaSorin Liaison</td>
<td>0,15</td>
<td>300</td>
</tr>
</tbody>
</table>
MAGLUMI Total PSA

Profilo di Precisione (range: 0.010 – 123 ng/mL)
Dati in File: Medical Systems

Totale risultati: n.343 replicati n.3 lotti di kit

Low level PSA:
5 pool testati 20 giorni con 1 lotto kit (100 risultati);
5 pool (veri sieri umani) testati in 3 replicati
per 5-6 sedute con 2 lotti kit (165 risultati);

Low, Mid and High PSA:
n.4 sieri umani, testati in 3 replicati,
indiluiti e con diluzioni seriali (78 risultati)

Sensibilità Funzionale
(Limit of Quantitation, LoQ, with a maximum CVt of 20%)

LoQ = 0.023
Test PSA: Sensibilità Funzionale e Intervallo di Misura

PSA Maglumi è l’unico test di 3° Generazione con Range Esteso
Sensibilità Funzionale PSA Immulite 2000 3G

Third Generation PSA

Analytical Sensitivity: 0.003 ng/mL

Functional Sensitivity: 0.01 ng/mL, as demonstrated by the studies summarized in the Precision section. (Functional sensitivity is defined as the lowest concentration that can be measured with an interassay CV of 20%.)
Correlazione kit PSA Maglumi vs test PSA Immulite 3G
Dati in File. Medical Systems S.p.A.

Comparison Chart

<table>
<thead>
<tr>
<th>Sensibilità</th>
<th>Funzionale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kit PSA</td>
<td>Imm.3G</td>
</tr>
<tr>
<td>(0,030)</td>
<td>(0,010)</td>
</tr>
</tbody>
</table>

\[ y = 0.7168 \times 0.948 \]
\[ R^2 = 0.9886 \]
\[ r = 0.9943 \]
Sensitivity
Functional

Functional sensitivity is defined as the lowest concentration that can be measured with an inter-assay coefficient of variation (CV) less than or equal to 20%. The calculated %CV for one reagent lot from all sites was plotted against the mean concentration of each panel. A parametric curve was fitted through the data, and the functional sensitivity was determined to be less than 0.05 ng/mL, which corresponded to less than 20% CV on the fitted curve.
Correlazione kit PSA Maglumi vs test PSA Architect
Dati in File. Medical Systems S.p.A.

Comparison Chart

Sensibilità Funzionale
Kit PSA Maglumi (0,030)

Sensibilità Funzionale
Kit PSA Architect (0,050)

$y = 0.9093x - 0.0055$
$R^2 = 0.9968$
$r = 0.9984$
Variability of assay methods for total and free PSA after WHO standardization.
Foj L, Filella X, Alcover J, Augé JM, Escudero JM, Molina R.

The variability of total PSA (tPSA) and free PSA (fPSA) results among commercial assays has been suggested to be decreased by calibration to World Health Organization (WHO) reference materials. To characterize the current situation, it is necessary to know its impact in the critical cutoffs used in clinical practice. In the present study, we tested 167 samples with tPSA concentrations of 0 to 20 μg/L using seven PSA and six fPSA commercial assays, including Access, ARCHITECT i2000, ADVIA Centaur XP, IMMULITE 2000, Elecsys, and Lumipulse G1200, in which we only measured tPSA. tPSA and fPSA were measured in Access using the Hybritech and WHO calibrators. Passing–Bablok analysis was performed for PSA, and percentage of fPSA with the Hybritech-calibrated access comparison assay. For tPSA, relative differences were more than 10% at 0.2 μg/L for ARCHITECT i2000, and at a critical concentration of 3, 4, and 10 μg/L, the relative difference was exceeded by ADVIA Centaur XP and WHO-calibrated Access. For percent fPSA, at a critical concentration of 10%, the 10% relative difference limit was exceeded by IMMULITE 2000 assay. At a critical concentration of 20 and 25%, ADVIA Centaur XP, ARCHITECT i2000, and IMMULITE 2000 assays exceeded the 10% relative difference limit. We have shown significant discordances between assays included in this study despite advances in standardization conducted in the last years. Further harmonization efforts are required in order to obtain a complete clinical concordance.

... The Access PSA assay using the Hybritech calibrators was selected as the reference assay because it was the original assay developed for the measurement of tPSA, and it was the first FDA-approved assay for prostate cancer detection ...
... For patients with tPSA of <2 μg/L, the slopes ranged from 0.882 to 1.098 for ADVIA Centaur XP and IMMULITE 2000 assays, respectively. The y-intercepts ranged from −0.0088 to 0.0030 μg/L for ARCHITECT i2000 and Lumipulse G1200 assays, respectively. The correlation coefficients ranged from 0.969 to 0.995. At the critical concentration of 0.2 μg/L, a relative difference of 10% was exceeded by ARCHITECT i2000.

... Results for percent fPSA for patients with tPSA between 2 and 20 μg/L and the Hybritech-calibrated Access comparison assay are shown in Fig. 4. The slopes ranged from 0.936 to 1.245 for IMMULITE 2000 and ARCHITECT i2000 assays, respectively. The y-intercepts ranged from −2.862 to 0.172 μg/L for ARCHITECT i2000 and Elecsys assays, respectively. The correlation coefficients ranged from 0.947 to 0.976. For the percentage of fPSA, at a critical concentration of 10%, the 10% relative difference limit was exceeded only by IMMULITE 2000 assay. At a critical concentration of 20 and 25%, ADVIA Centaur XP, ARCHITECT i2000, and IMMULITE 2000 assays exceeded the 10% relative difference limit (Table 4).

... The reference range of 4 μg/L was originally determined for the Hybritech Tandem-R assay [15] and later ratified, using the same test, by Catalona et al. [16] in a large multicenter study. This cutoff has been used to select patients for biopsy in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial [17] and in the European Randomized Study of Screening for Prostate Cancer until 1997, when all the centers involved in this study recommended biopsy in men with PSA of ≥3 μg/L [18]. Both studies used the Hybritech PSA test, currently manufactured by Beckman Coulter.

These critical points have been used also for other tests, even when there are differences between assays.
According to our data, differences higher than the limit bias of 10% at the critical points of 3 and 4 μg/L were observed for ADVIA Centaur and WHO-calibrated Access. On the other hand, differences between 5 and 10% at these critical points were observed for ARCHITECT 2000.

Differences in tPSA values were lower for patients with PSA between 0 and 2 μg/L. This is a decisive range of results with usefulness in following patients treated with radical prostatectomy. Nevertheless, there is a high degree of variability in the definition of biochemical recurrence after radical prostatectomy, and the PSA cutoffs of 0.2 and 0.4 μg/L are the most commonly points used [20]. We have chosen these critical points to evaluate differences between assays. Relative differences higher than 10% were observed only for ARCHITECT at the critical point of 0.2 μg/L. However, differences between 5 and 10% were observed for three and five assays at the cutoffs of 0.2 and 0.4 μg/L, respectively (Table 2).

...We studied the variability of percent fPSA using six different assays, especially considering the cut points of 10, 20, and 25%. Relative differences higher than 10% in reference to the Hybritech-calibrated Access were observed for the majority of assays, especially at the cutoffs of 20 and 25%. Differences lower than 5% were observed only for Elecsys at the three critical points evaluated in our study. Initial results comparing Access PSA test using WHO and Hybritech calibrators showed that results of tPSA are 20–25% lower when WHO standard is adopted [21]. This trend is also observed for other assays using the reference material from WHO, but for other assays, differences with the Hybritech PSA test are minimal despite the use the WHO standard. Discordant PSA and percent fPSA results between assays are maintained despite the introduction of WHO standards for the measurement of both biomarkers. Differences between PSA assays using WHO standards may be due to other factors, including matrix effects and affinity antibodies.

...We have shown in our study that differences between assays could be considerable. We obtained that the maximum differences for tPSA could be over 20% in 74% of patients and over 40% in 41% of patients.
Method Comparison for Determination of the Tumor Markers
AFP, CEA, PSA and Free PSA Between Immulite 2000 XPI and Dimension Vista 1500

Method comparison for determination of the tumor markers AFP, CEA, PSA and free PSA between Immulite 2000 XPI and Dimension Vista 1500.

... The assays for tPSA and fPSA, as developed with the LOCI technology for the Dimension Vista, show good comparability with results obtained from the IMMULITE 2000 XPI. However, lower measurement ranges for fPSA as well as individual divergences must be taken into consideration in the event of method changeover.

... For the entire measuring range, correlation of Vista PSA and IMMULITE 2000 PSA (N = 711) was at r = 0.971, slope 1.006 (1.000 - 1.013, 95% CI), intercept 0 (-0.003 – 0.95% CI) (Figure 4). In the measuring range relevant for clinical diagnosis, <20 ng/mL, correlation of both tests was very good (r = 0.994) ... A changeover from IMMULITE 2000 PSA to Vista PSA would not necessarily result in misinterpretations since we found no significant differences in the method comparison, not even in the generally defined cut-off value of 4 ng/mL [27].

... For the entire measuring range, correlation of Vista free PSA and IMMULITE 2000 free PSA (N = 95) was at r = 0.988, slope 0.910 (0.886 - 0.949, 95% CI), intercept 0.007 (-0.005 - 0.018, 95% CI) (Figure 5) ... Correlation between Vista free PSA and IMMULITE 2000 free PSA was very good. However, values were slightly lower across the entire measurement range (Figure 5).

... Our method comparison with IMMULITE 2000 delivered convincing results. However, in individual cases, markedly different values were obtained. Therefore, parallel measurements, as required in all tumor marker changeovers, are essential in the event of instrument changeover.
Assessing the necessity of including a crossover period with dual reporting when changing total prostate-specific antigen methods.

Rutledge AC, Pond GR, Hotte SJ, Kavsak PA.

... Protein tumor marker results do not agree well across platforms from different manufacturers when immunoassays are not standardized. Accordingly, it is recommended that laboratories have a specific protocol in place when changing tumor marker platforms [1]. One strategy is to run the current and the new methods in parallel for a period of time and to report results from both tests (i.e., dual reporting). This permits new baseline values to be established.

... Options are limited for the laboratory when changing analytical methods for tumor markers. Standardization between methods should alleviate this burden. Our objective was to assess the necessity of dual reporting for total prostate-specific antigen (tPSA) when changing methods that have been calibrated against the World Health Organization PSA reference material.

... Despite the WHO primary reference material for PSA, a reference measurement procedure is still lacking for tPSA [7]. This deficiency has prevented tPSA results from different platforms from being used interchangeably, with differences of up to 15% being observed [2,3]. The lack of interassay agreement in tPSA results has also been noted to affect clinical interpretation [8].
... Based on the Passing & Bablok regression and Pearson correlation, the data from the initial validation and the crossover period appear to agree and correlate very well between the Roche E-Modular and Abbott ARCHITECT tPSA assays.

However, when assessing comparability of tPSA results using the CLSI guideline [5] (Method#1), only half of the results are in agreement. Using this criterion of comparability based on CVi, it would be prudent to perform dual reporting when changing tPSA methods. The dual reporting would ensure that the total PSA results from the new method could be placed in context by the results from the current method, thereby avoiding confusion and possible misinterpretation by clinicians.

Using an alternative approach (Method#2), based on analytical imprecision, a metric the laboratory is accustomed to assessing and discussing, a concentration range of optimal agreement between the two tPSA assays may be empirically determined. With as few as 40 samples covering a sufficient span of tPSA results, a concentration range where agreement is most acceptable may be observed. In our validation study, the range of concentrations where the most consecutive Roche results fell within the allowable imprecision of the Abbott tPSA assay was 3.3 to 19 μg/L. Importantly, testing this range in the crossover period confirmed that 3.3 to 19 μg/L yielded an optimal agreement range where 95% of the 331 paired results in this region were in agreement.

If only samples falling outside of the range of optimal agreement were tested on the current platform as well as the new platform, in this case only 70% of the samples would have required dual reporting.
It is important to emphasize that although the differences observed between the Roche and Abbott tPSA assays are most often not clinically relevant, there a number of instances where the variations alone may affect clinical decision making.

... For example, many would argue that the difference between a value of 2.9 and 4.2 μg/L (an identified discordant result in our crossover study) is minimal and unlikely to be clinically relevant. However, if a patient had monthly tPSA concentrations of 2.1, 2.3, 2.6, and then 4.2 μg/L, it would likely become clinically actionable given the rapid rise and doubling time, whereas concentrations of 2.1, 2.3, 2.6, then 2.9 μg/L might still warrant a wait and see approach. As if often the case, the laboratory is unaware of the clinical rationale on ordering a tPSA, as it may be used for monitoring therapy, detecting recurrence and possibly for screening [9], as such it is difficult to truly assess what impact an inaccurate tPSA result would have on patient care.

... Recommendations on how to best change tumor marker assays are limited; however, methods that have undergone some form of standardization, such as tPSA, should agree much better between platforms than the other tumor markers. Therefore, for tPSA, it may be possible to restrict dual reporting to concentrations outside an optimal agreement range. The approaches outlined here are unlikely to be useful for other tumor markers, which have not undergone standardization efforts and are likely to need complete dual reporting. It is important to note that while partial dual reporting would help both budgetary and workload issues, until a study assessing the impact of such an approach on clinical care is conducted, laboratories should still consider performing dual reporting of tPSA when changing methods so that clinicians can re-establish baseline values.

... These findings suggest that dual reporting of tPSA is required when changing methods. However, the extent of dual reporting may be limited to specific concentration ranges with future studies required to validate selective dual reporting when changing tPSA assays.
Forme molecolari di PSA

- Free PSA
  - Uncomplexed PSA (~10–15% of immunoreactive PSA in serum)

- PSA-ACT
  - $a_1$-antichymotrypsin-bound PSA
    - (~85–90% of immunoreactive PSA in serum)

- PSA-A2M
  - $a_2$-macroglobulin-bound PSA
    - (“occult”, i.e. not currently measurable)
Il primo test al mondo

Free PSA in AUTOMAZIONE

prodotto da DPC

(Diagnostic Products Corp, USA)

è stato rilasciato alle vendite nel 1994
Free to total PSA (F/T) ratio for distinguishing benign prostatic hyperplasia from prostate cancer.

Biomedecine & Pharmacotherapy Vol.50, 8, 1996, 416

Melone F, Muraro G, Maggio A.

Metodi utilizzati:

IMMULITE Free PSA
IMMULITE 3G PSA
Sw ProSTAT Medical Systems
Use of the Free/Total PSA Ratio in the Management of Prostatic Disease


Analytical Methods
Total PSA: IMMULITE Third Generation PSA (DPC, Los Angeles, US).

Data Processing
All results were processed using the "ProSTAT" software recently developed by Medical Systems SpA (Genoa, Italy) for calculating diagnostic efficiency and cost-effectiveness in prostatic diseases. The ProSTAT is an automated Microsoft Excel (version 5.0 or higher) for Windows template, which can generate real-time interactive statistics.

Results and Discussion
The following table summarizes the statistical evaluation of the data for all 313 patients.

<table>
<thead>
<tr>
<th></th>
<th>Total PSA</th>
<th>Free PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>259</td>
<td>259</td>
</tr>
<tr>
<td>Mean</td>
<td>3.9</td>
<td>20.8</td>
</tr>
<tr>
<td>Median</td>
<td>1.6</td>
<td>18.7</td>
</tr>
<tr>
<td>SD</td>
<td>4.2</td>
<td>10.9</td>
</tr>
<tr>
<td>SE</td>
<td>0.3</td>
<td>0.68</td>
</tr>
<tr>
<td>25th Percentile</td>
<td>0.63</td>
<td>13.9</td>
</tr>
<tr>
<td>75th Percentile</td>
<td>5.7</td>
<td>24.8</td>
</tr>
</tbody>
</table>

ROC curve analysis showed that the F/T PSA ratio cutoff value giving the best compromise between sensitivity and specificity is 11%. So we used this as the cutoff for the F/T PSA ratio and 4.0 ng/mL as the cutoff for total PSA in the ProSTAT analysis, which allows for evaluating the clinical significance of the two tests either separately or combined.

The four pathological criteria we introduced were:

- Criterion 1: malignant disease for PSA > 4.0 ng/mL.
- Criterion 2: malignant disease for F/T PSA < 11.
- Criterion 3: malignant disease for PSA > 4.0 ng/mL and F/T PSA < 11.
- Criterion 4: malignant disease for PSA > 4.0 ng/mL and F/T PSA < 11.

The evaluation results are described in the following table.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positives</td>
<td>50</td>
<td>47</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>True Negatives</td>
<td>176</td>
<td>228</td>
<td>160</td>
<td>244</td>
</tr>
<tr>
<td>False Positives</td>
<td>83</td>
<td>31</td>
<td>99</td>
<td>15</td>
</tr>
<tr>
<td>False Negatives</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>313</td>
<td>313</td>
<td>313</td>
<td>313</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>93%</td>
<td>87%</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>Specificity</td>
<td>68%</td>
<td>88%</td>
<td>62%</td>
<td>94%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>38%</td>
<td>60%</td>
<td>35%</td>
<td>74%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>98%</td>
<td>97%</td>
<td>100%</td>
<td>96%</td>
</tr>
<tr>
<td>Diagnostic Efficiency</td>
<td>72%</td>
<td>88%</td>
<td>68%</td>
<td>92%</td>
</tr>
</tbody>
</table>

The data analysis shows that the F/T PSA ratio represents an improvement in the diagnosis of prostate disease when compared to total PSA. The F/T PSA ratio by itself (criterion 2) yields an increase in diagnostic efficiency (88% compared to 72%); but the best results are obtained by combining the F/T PSA ratio with total PSA (criterion 4). In this way, we achieved a diagnostic efficiency of 92% and very good sensitivity, specificity, positive predictive value and negative predictive value scores.

Metodi utilizzati:
IMMULITE Free PSA
IMMULITE 3G PSA
Sw ProSTAT Medical Systems SpA


Use of the Free/Total PSA ratio in the management of prostatic disease.
International Conf. PSA & Prostatic Disease.
May 21-22, 1996 Llanberis, Wales UK
ZB143-B 1996, A11, 45
11 Use of the Free/Total PSA Ratio in the Management of Prostatic Disease


Free PSA IMMULITE - Aimo et al, 1996

Metodi utilizzati:
IMMULITE Free PSA
IMMULITE 3G PSA
Sw ProSTAT Medical Systems SpA

Use of the Free/Total PSA ratio in the management of prostatic disease.
International Conf. PSA & Prostatic Disease.
May 21-22, 1996 Llanberis, Wales UK
ZB143-B 1996, A11, 45
Correlazione PSA Maglumi vs test Roche Elecsys
Dati in File, Medical Systems S.p.A.

Coefficiente di correlazione: $r = 0,9954$
Campioni: n. 60
Correlazione Free PSA Maglumi vs test Roche Elecsys
Dati in File, Medical Systems S.p.A.

Coefficiente di correlazione: $r = 0,9854$
Campioni: n. 60

$y = 0,9315x - 0,0255$
$R^2 = 0,971$
Comparison Chart

Total PSA range = 2–16 ng/mL

Snibe Maglumi Ratio F/T PSA %

Roche Cobas Ratio F/T PSA %

$y = 0.8451x + 2.1485$

$R^2 = 0.9034$

$r = 0.9505$
Quando dosare il Free PSA per usare il Ratio F/T PSA% ?

**Diagnosi Differenziale con Malattia Benigna e Indicazione all’Esecuzione di Biopsia Prostatica:**

- **Raccomandato per valori PSA >4 e <10 ng/mL**  
  (Riff. 1, 2, 3, 4, 5)
- Proposto per valori PSA >2.0 e <10 ng/mL  
  (Riff. 6, 7)
- Proposto per valori PSA >2.5 e <10 ng/mL  
  (Riff. 4, 8)
- Proposto per valori PSA >3.0 e <10 ng/mL  
  (Riff. 9)
- **Clinicamente inutile per valori PSA >10 ng/mL**  
  (Riff. 3)
- **Mai per valori PSA <2.0 e >10 ng/mL**  
  (Riff. 1-9)

**Monitoraggio di Carcinoma Prostata e Dopo Prostatectomia Radicale:**

- **Clinicamente inutile**  
  (Riff. 3)

2. Guida all’uso clinico biomarcatori in oncologia. Gion M et al. Biochimica Clinica, 2011
3. Guidelines Prostate Cancer, European Association of Urology, EAU Guidelines 2010
4. Linee Guida Nazionali Carcinoma Prostata, Agenzia Servizi Sanitari Regionali, AgeNaS
5. Linee Guida Carcinoma della Prostata, Associazione Italiana di Oncologia Medica, AIOM
7. Linee Guida Carcinoma della Prostata, Regione Piemonte, 2009
8. Linee Guida Biopsia Prostatica. Società Italiana di Urologia Oncologica, SIURO, 2005
PSA Reflex, Delibere Regionali:

Mai dosaggio del PSA Libero per valori di PSA <2.5 e >10 ng/mL (Rif. 1)

Mai dosaggio del PSA Libero per valori di PSA <2.0 e >10 ng/mL (Riff. 2,3)

1. Regione Emilia-Romagna (Deliberazione Giunta Regionale N.1779 del 22/11/2010)

Nota: nel paziente che è stato sottoposto a prostatectomia la prestazione da richiedere è il solo PSA totale. In questo caso, infatti, rilevare la presenza di PSA dopo l’intervento è in ogni caso indice di presenza di tessuto prostatico residuo, indipendentemente dalla concentrazione del PSA libero. La recidiva è, infatti, eventualmente indicata dalla ripresa della secrezione del PSA e non dalla quota non legata alle proteine vettrici.

2. Regione Lombardia (Deliberazione Giunta Regionale N.IX/2057 del 28/07/2011)