



Prostate Cancer Gene 3 (PCA3):

the new tool available **to improve the diagnosis**
of prostate cancer in a simple urine test.*



PROGENSA™
PCA3

* CE



PROGENSA™ PCA3

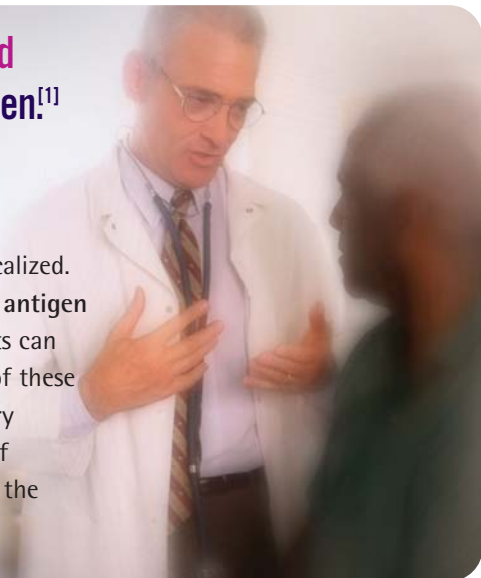
can provide
critical information
for better management
of patients concerned
about prostate cancer.

- ▶ **PCA3** is highly specific to PCa, unlike serum PSA which may be elevated due to a number of benign conditions
- ▶ **PCA3** is non-invasive and gives insight into the prostate cell composition, unlike biopsy which is invasive and subject to missing malignant cells through sampling error
- ▶ **PROGENSA™ PCA3** assay uses first catch urine immediately following a good urologic DRE
- ▶ **PROGENSA™ PCA3** assay improves the diagnosis of PCa, helping reduce the number of unnecessary biopsies

Prostate cancer (PCa) is the second most commonly diagnosed malignancy and a leading cause of cancer death in European men.^[1]

Survival of patients who receive a diagnosis of early-stage disease is substantially better than that of patients who receive a diagnosis of late-stage disease.^[2-3]

However, in most patients, PCa symptoms do not develop when the tumour is still localized. Consequently, today early diagnosis of PCa relies heavily on serum prostate specific antigen (PSA) concentration and digital rectal examination (DRE). The outcome of both tests can trigger the performance of a prostate biopsy to confirm the diagnosis of PCa. Each of these three measures has shortfalls which contribute to an increased number of unnecessary biopsies. There is a need for an additional diagnostic test, which can help determine if additional biopsies are required. PROGENSA™ PCA3 is a new tool available to improve the diagnosis of prostate cancer in a simple urine test.



The dilemmas when using DRE, PSA and prostate biopsy in the diagnosis of prostate cancer

DRE dilemma

DRE is considered a standard diagnostic tool in the diagnosis of PCa. Nevertheless, it is recognized that the positive predictive value (PPV) for diagnosing PCa is very low (10–19% in this patient population).^[4] In addition, the reproducibility of a DRE is poor and inter-examiner variability high.^[4] DRE remains an important part of early PCa diagnosis, however these shortcomings underscore the need for additional diagnostic measures.

PSA dilemma

PSA is another standard tool in the diagnosis of PCa. PSA levels in the blood increase when the prostate gland enlarges. Unfortunately, several common conditions that are not malignant can result in elevated PSA levels, such as benign prostatic hyperplasia (LUTS/BPH) and prostatitis. In other words, PSA is not PCa-specific. Therefore, the PPV of PSA in a concentration up to 10 ng/mL is rather low (approximately 20–30%).^[5-6] Serum PSA remains a useful and cost-effective test, however, a lack of specificity for PCa underscores the need for additional diagnostic information.

Current dilemmas in the diagnosis of PCa

- ▶ An increased PSA concentration and an abnormal/suspicious DRE are both triggers to perform a prostate biopsy
- ▶ The PPV of both DRE (<20%) and PSA (<30% at concentrations below 10 ng/mL) is far from optimal
- ▶ A biopsy examines approximately 1% of prostate tissue and as many as 10–25% of patients with a negative biopsy have Pca^[7]
- ▶ Prostate biopsy is not only costly, but also induces distress and pain/discomfort for the patient and can be associated with complications that are sometimes severe
- ▶ There is need for an additional diagnostic test to better predict biopsy outcome and reduce the number of unnecessary biopsies

Prostate biopsy dilemma

Transrectal ultrasound (TRUS)-guided biopsy is the mainstay of PCa diagnosis. However, due to the low PPV of DRE and PSA, a large percentage of an increasing number of men will undergo an unnecessary initial biopsy depending on the PSA cut-off utilized.^[6] This will only grow due to the increased tendency for screening and the use of lower PSA thresholds.

A negative biopsy result can lead to a false sense of security as only 1% of total prostate tissue is examined as a result of the biopsy, there exists a good possibility that some cancer is missed (current estimate 15–20%).^[10] Approximately 10–25% of men with PCa remain undiagnosed after a single set of sextant biopsies.^[7]

The high percentage of initially negative and subsequent repeat biopsies will induce considerable costs for society and negatively impact on the patients' quality of life. The prevalence rates of discomfort and pain and the 3 most common complications (haematuria, haematospermia and rectal bleeding) reported in prospective studies involving at least 100 patients with 6–12 cores vary considerably (Table 1).

Taking all these factors into consideration, there is obviously a need for an additional diagnostic test that has a greater PPV and, consequently, better identifies the patients who will indeed have a positive biopsy. This should reduce the number of initial and repeat biopsies.

Most common complications following prostate biopsy

| Complication: | % of patients |
|-----------------|---------------|
| Discomfort | 34-70% |
| Pain | 50-70% |
| Haematuria | 10-74% |
| Haematospermia | 10-78% |
| Rectal bleeding | 1-40% |

Table 1: Prevalence (% of patients) of most common complications following 6-12 core prostate biopsy (range reported in prospective studies involving at least 100 patients)^[5,6,7,8,9,10,11,12,13]

PCA3 and its role in improving the diagnosis of prostate cancer

Introduction to PCA3

Prostate Cancer Gene 3 (PCA3; in the past also referred to as DD3) is the first molecular diagnostic assay that can contribute to solving the mentioned clinical dilemmas and improve the diagnosis of PCa. In contrast to PSA, PCA3 is not only prostate-specific, but also PCa-specific (Figure 1).^[14] Moreover, in contrast to PSA, the PCA3 gene is highly over-expressed (median 66-fold) in >95% of PCa tissue compared to normal or benign prostate tissue of the same patients.^[14,15] As PCA3 is more PCa-specific than PSA, the PPV of the PCA3 assay to diagnose PCa is almost twice that of serum PSA (75% vs. 38%).^[16]

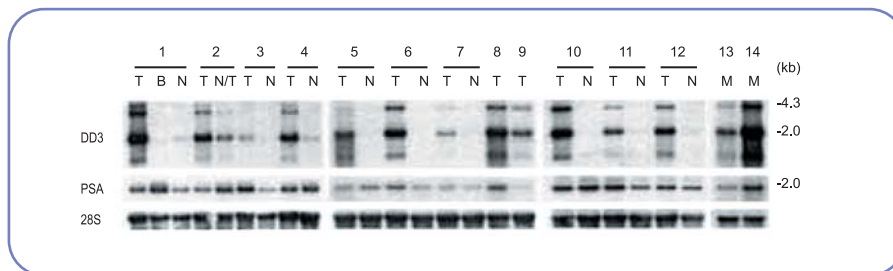


Figure 1: PCA3 (DD3) mRNA is only expressed in prostatic tumour (T and M) and not in benign (B) or normal (N) tissue, in contrast to PSA mRNA^[14]

Using the PROGENSA™ PCA3 assay in clinical practice

The PROGENSA™ PCA3 assay from Gen-Probe^[17] detects the presence of PCA3 mRNA from whole urine and is easy to use (Figure 2):

1. Perform DRE (3 strokes per lobe) in order to have a sufficient number of prostate cells in the urine.
2. After the DRE, collect 20–30 mL first-catch urine from the patient.
3. Transfer urine within 15 minutes to the transport tube, fill until the fluid level is between the black fill lines.
4. Follow the sample storage guidelines on the transport tubes provided by the testing laboratory.

Note: The transport tubes can be obtained from a PROGENSA™ PCA3 testing laboratory

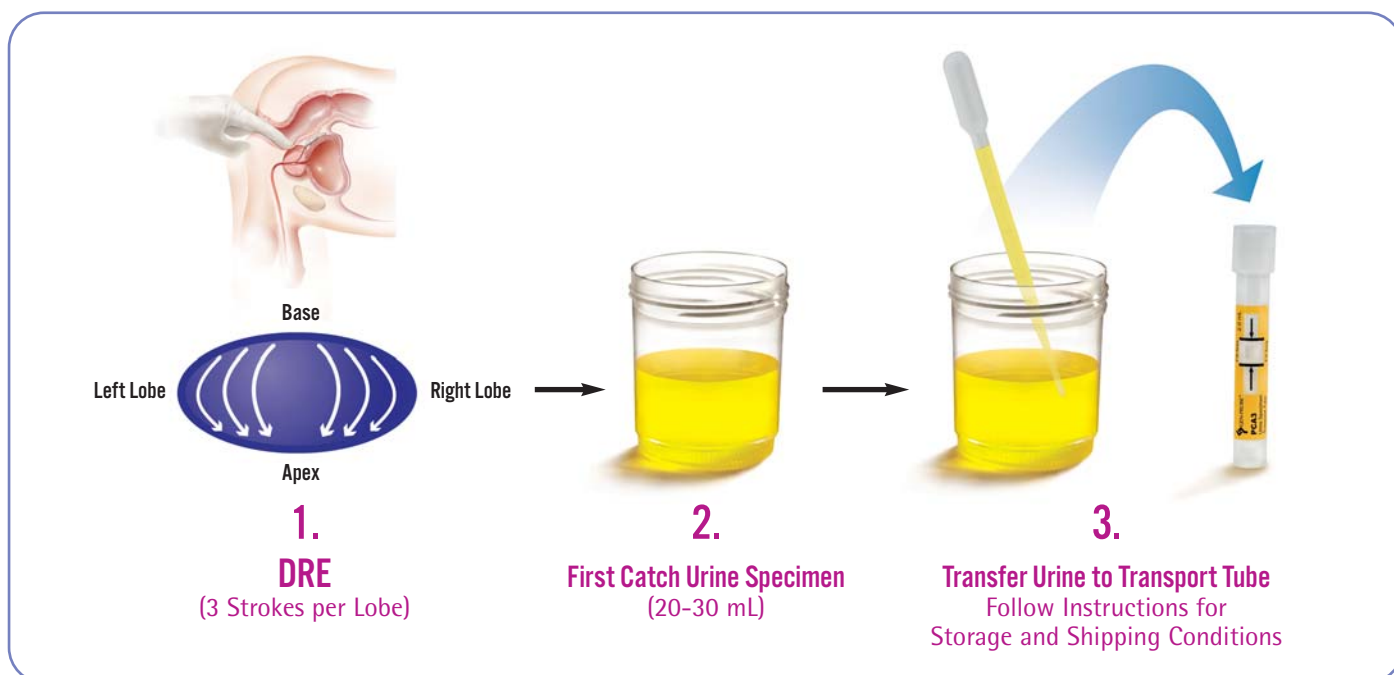


Figure 2: The PCA3 urine specimen collection procedure
 DRE Instruction: apply pressure on the prostate, enough to depress the surface approximately 1 cm, from the base to the apex and from the lateral to the median line for each lobe and repeat this 3 times

Using the PCA3 assay result for improving the diagnosis of prostate cancer

Calculating the PCA3 score

Using transcription-mediated amplification (TMA) technology, PCA3 and PSA mRNA molecules are amplified and the PCA3 score is calculated. PCA3 and PSA mRNAs are quantified, and the PCA3 Score is determined based on the ratio of PCA3/PSA mRNA. In addition to normalizing PCA3 signal, measurement of PSA mRNA also serves to confirm that the yield of prostate-specific RNA is sufficient to generate a valid result. Higher PCA3 scores correlate with higher probability of a positive prostate biopsy. The PCA3 score = $1000 \times [\text{mRNA PCA3}] / [\text{mRNA PSA}]$.

PCA3 score in men scheduled for prostate biopsy

The PCA3 score was determined in 529 men (mean age 64 years) with mean serum PSA 7.9 ng/mL.^[24] Biopsies were positive for 180 men (34%). The higher the PCA3 score, the higher the percentage of men with a positive biopsy (Figure 4). A PCA3 score of 35 provided the greatest diagnostic accuracy, i.e., balance between sensitivity (53%) and specificity (74%). The PCA3 assay outcome is considered positive if the PCA3 score is ≥ 35 . A patient with a PCA3 score ≥ 35 has a high probability of having PCa. If the outcome is negative (PCA3 score < 35), the patient has a lower probability of having PCa. Decisions to biopsy or pursue alternative courses should be made in consideration of all patient history.



Negative Biopsy Result in enlarged prostate group

Average TRUS = 67.7 cc
Average PSA = 7.5 ng/mL
Average serum PCA3 score = 32.6
n = 154

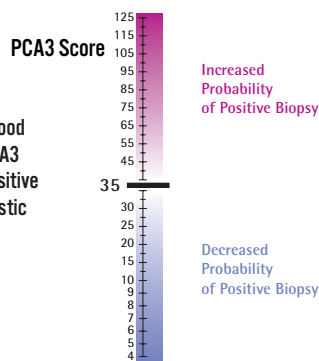


Positive Biopsy Result in normal size prostate group

Average TRUS = 29.2 cc
Average PSA = 7.0 ng/mL
Average serum PCA3 score = 58.9
n = 144

Figure 3: Comparing sub-populations based on Biopsy result and TRUS prostate volume (Enlarged = 45+, Normal = < 45) average PCA3 score increases significantly in positive biopsy result group, while average PSA actually goes down slightly for positive group.

Figure 4: As the PCA3 score increases the likelihood for positive biopsy increases. As the PCA3 score decreases, the likelihood for a positive biopsy decreases. The greatest diagnostic utility occurs at a cut-off of 35.



A PCA3 score of approximately 35 is also able to differentiate normal men (aged < 45 years with no known PCa risk factors) from men with LUTS/BPH, untreated PCa and PCa treated with radical prostatectomy. There was no overlap in the 95% confidence intervals (CIs) between the groups ($P < 0.01$) (Figure 5).^[19]

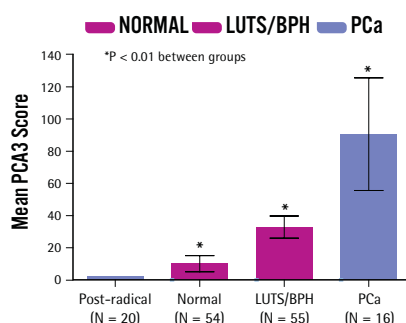


Figure 5: A PCA3 score of approximately 35 also seems to be able to differentiate between normal/benign vs. malignant prostate tissue^[19]

In the same population, the mean serum PSA concentration increased with larger prostate volumes whereas the mean PCA3 score was not influenced by prostate volume. This suggests that PCA3 is independent of prostate volume and therefore more PCa-specific than PSA (Figure 6).^[20]

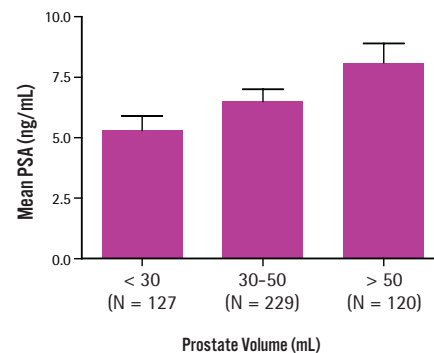
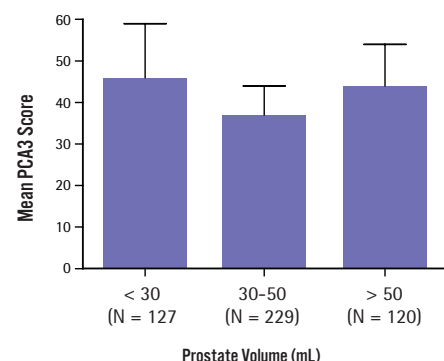


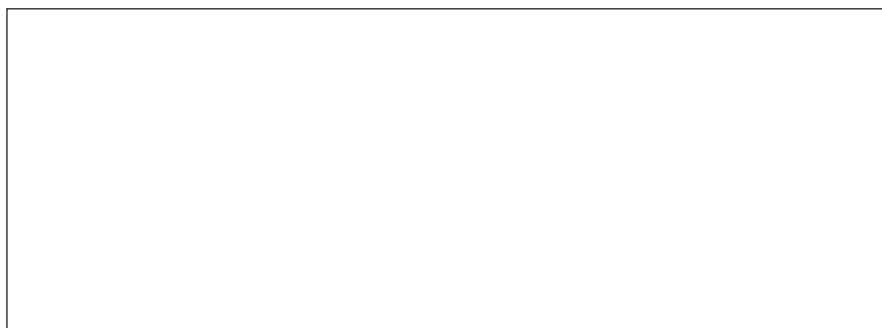
Figure 6: Whereas serum PSA (mean + 95% CI) clearly increases with prostate volume, the PCA3 score (mean + 95% CI) is independent of prostate volume^[20]



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