Performance Characteristics

AdnaTest ProstateCancerSelect, cat. no. T-1-520 and *AdnaTest ProstateCancerDetect*, cat. no. T-1-521

Version management

This document is the *AdnaTest ProstateCancerSelect/Detect* Performance Characteristics, Version 1, R1.

	Check availability of new electronic labeling revisions at **www.qiagen.com/HB-2099** and **www.qiagen.com/HB-2100** before test execution.

Recovery

Two cultured LnCap prostate cancer cells were spiked into blood samples from healthy donors to determine the recovery rates achieved with *AdnaTest ProstateCancerSelect/Detect* (Table 1).

Table 1. *AdnaTest* recovery rate of tumor cells spiked into blood samples from healthy donors

	Recovery (%)	Number of samples
Two tumor cells spiked into 5 ml blood	95	40

The recovery rate is 95% for detection of 2 tumor cells spiked into 5 ml of blood from healthy donors.

Specificity

AdnaTest ProstateCancerSelect/Detect was used to analyze 40 healthy donors to determine the rate of false positives at the given cut-off (0.10 ng/µl fragment concentration for each gene profile included, except for actin).

Table 2. Determination of specificity

Controls	Specificity (%)	Number of samples
Healthy donors	100	40

AdnaTest ProstateCancerSelect/Detect demonstrated a specificity of 100% (Table 2).

Reproducibility

Twenty blood samples from healthy donors were spiked with 10 LnCap prostate cancer cells per sample. Blood samples were analyzed by two operators using *AdnaTest ProstateCancerSelect/Detect* to determine the reproducibility. The intra-assay and the inter-assay reproducibility were 100% (Table 3).

Table 3. Reproducibility of *AdnaTest ProstateCancer Select/Detect*

Operator	Positive *AdnaTest* result/samples	Intra-assay reproducibility (%)	Inter-assay reproducibility (%)
A	10/10	100	100
B	10/10	100	100

Precision

To determine the precision, aliquots of cDNA were pooled and analyzed using *AdnaTest ProstateCancerDetect*. Two operators analyzed 30 cDNA samples, consisting of 3 independent measurements of 10 samples. The intra-assay and inter-assay precision were 100% (Table 4).

Table 4. Precision of *AdnaTest ProstateCancerDetect*

Operator	Positive *AdnaTest* result/samples	Intra-assay precision (%)	Inter-assay precision (%)
A	30/30	100	100
B	30/30	100	100

Interfering substances

Anticoagulants

When drawing and transporting blood, use of anticoagulants is mandatory. However, heparin and citrate lead to aggregate formation after addition of *AdnaTest* immunomagnetic beads, which can result in a lack of test results or false test results. However, EDTA and ACDA (citrate/dextrose/adenine solution A) are compatible with *AdnaTest* immunomagnetic beads.

Hemolysis

Hemolysis in blood samples (plasma fraction appears red) is, in most cases, due to incorrect transportation or storage conditions. Such samples may give false-negative results and should be discarded.

Chemotherapeutics, targeted therapy drugs and anti-hormonal regimens

Chemotherapeutics (taxanes, cisplatin, oxaliplatin, 5-FU, anthracycline, irinotecan etc.) are potent cytotoxins and cause damage or rapid cell death in a blood sample. This results in a high likelihood of false-negative results when using *AdnaTest* immunomagnetic beads. After administration of these substances, the human body needs around 5–7 days to detoxify them (Table 5). Blood samples drawn during this time must not be used with *AdnaTest* immunomagnetic beads.

Table 5. Half-lives of chemotherapeutics

Drug	Half life	Reference
5-Fluouracil	Up to 20 minutes	**www.drugs.com/pro/fluorouracil-injection.html**
Docetaxel	Up to 11.1 hours	**www.drugs.com/pro/docetaxel.html**
Cis-platinum	Up to 30 minutes	**www.drugs.com/pro/cisplatin.html**
Carbo-platinum	Up to 5.9 hours	**www.drugs.com/pro/carboplatin.html**
Paclitaxel	Around 25.4 hours	**www.drugs.com/pro/paclitaxel.html**

The same precaution is also recommended for targeted therapy regimens such as antibodies (Herceptin®, bevacizumab, cetuximab etc.), tyrosine kinase blockers (olaparib, Iressa®, Erbitux®, lapatinib etc.) and anti-hormonal drugs (tamoxifen, abiraterone, enzalutamide etc.) administered as a single drug or in combination with chemotherapeutics.

In clinical trials demonstrating the prognostic value of circulating tumor cells (CTC) identified and characterized using *AdnaTest* immunomagnetic beads, no negative interference of chemotherapeutics, targeted therapies or anti-hormonal therapies was observed, provided the waiting period of at least 7 days after administration of the drug was complied with. Furthermore, a negative impact of common co-medications (Aspirin, ibuprofen, aprepitant, steroids etc.) is unlikely but is being monitored.

Interfering conditions

Blood clotting

In the context of clinical trials, we observed blood clotting after incubation with *AdnaTest* immunomagnetic beads – most frequently in blood samples from patients in a late disease state. Blood samples that exhibit clotting are difficult to process during the *AdnaTest* workflow due to increased viscosity and are difficult to pipet. They also contain an unacceptably high number of contaminating leukocytes, which leads to false-positive results. Such samples must be discarded.

Benign organic disease and chronic inflammatory conditions

Benign organic disease and chronic inflammation, such as arthritis, benign prostatic hyperplasia (BPH), Crohn's disease etc., do not lead to false-positive *AdnaTest* results.

Acute allergy

With acute allergic conditions, there is an increased number of contaminating leucocytes after CTC enrichment using *AdnaTest* immunomagnetic beads. Therefore, false-positive results cannot be fully excluded.

Clinical studies

A total of 12 patients with metastatic castrate-resistant prostate cancer (CRPC) were followed during docetaxel treatment. A first sample was analyzed at baseline and 2 further samples were analyzed during follow-up.

With regards to Androgen Receptor (AR) activation, it was clearly demonstrated that AR activation and deactivation correlated strongly with the rate of CTC elimination due to therapeutic intervention. However, the CTC positivity rate dropped during the course of therapy from 70% at baseline to ~35% during follow-up and AR positivity decreased from 55% to ~11%. Due to the therapy, AR-positive CTC subclones are more affected by docetaxel treatment than AR-negative CTC. These findings correlate well with those from Darshan et al. 2011, in which a taxane-induced blockade of AR nuclear transport and signaling was observed.

These findings indicate the specific and sensitive detection of CTCs in prostate cancer clinical samples as well as an assessment of genetic profiles related to therapeutic targets.

Reference

Darshan, M.S. et al. (2011) Taxane-Induced Blockade to Nuclear Accumulation of the Androgen Receptor Predicts Clinical Responses in Metastatic Prostate Cancer. Cancer Res. 2011 Sep 15; **71(18)**: 6019–6029. Published online 2011 Jul 28. doi: 10.1158/0008-5472.CAN-11-1417.

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