Performance Characteristics

AdnaTest BreastCancerSelect, cat. no. T-1-508 and *AdnaTest BreastCancerDetect*, cat. no. T-1-509

Version management

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

	Check availability of new electronic labeling revisions at **www.qiagen.com/HB-2109** and **www.qiagen.com/HB-2110** before test execution.

Recovery

Two and 5 cultured MCF7 breast cancer cells were spiked into blood samples from healthy donors to determine the recovery rates achieved with *AdnaTest BreastCancerSelect/Detect* (Table 1).

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

Breast cancer	Two cells	Five cells
Positive	159	168
Negative	16	3
Total	175	171
Recovery	**91%**	**98%**

The recovery rate is 91% for detection of 2 tumor cells spiked into 5 ml of blood from healthy donors. Five cells in 5 ml of blood from healthy donors can be successfully detected in 98% of all cases.

Specificity

AdnaTest BreastCancerSelect/Detect was used to analyze 233 healthy donors to determine the rate of false positives at the given cut-off (0.15 ng/µl fragment concentration for each gene profile included, except for actin).

Table 2. *AdnaTest* specificity

Breast cancer	Healthy donors
Positive	7
Negative	226
Total	233
Specificity	**97%**

This demonstrated a specificity of 97% for *AdnaTest BreastCancerSelect/Detect* (Table 2).

Reproducibility

Twenty blood samples from healthy donors were spiked with 10 MCF-7 breast cancer cells per sample. Blood samples were analyzed by two operators using *AdnaTest BreastCancer-Select/Detect* to determine the reproducibility. The intra-assay and the inter-assay reproducibility were 100% (Table 3).

Table 3. Reproducibility of *AdnaTest BreastCancer 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 BreastCancerDetect*. 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 *AdnaTestBreastCancerDetect*

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 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 due to increased viscosity during the *AdnaTest* workflow 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 prostate 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 leukocytes after circulating tumor cell enrichment using *AdnaTest* immunomagnetic beads. Therefore, false-positive results cannot be fully excluded.

Clinical validation

Overall survival analysis based on circulating tumor cells in metastatic breast cancer

In a performance evaluation study conducted at the Clinics for Gynecology and Obstetrics, University of Essen, Germany (Tewes et al. 2009), patients with metastatic breast cancer were tested using *AdnaTest* immunomagnetic beads and followed up during therapy. Of 32 patients enrolled to date, circulating tumor cells persisted during therapy in 50% (16/32) of patients. In an analysis of overall survival, a marked difference in survival for the *AdnaTest* positive and *AdnaTest* negative cohorts was observed – log-rank p=0.005 (Figure 1).

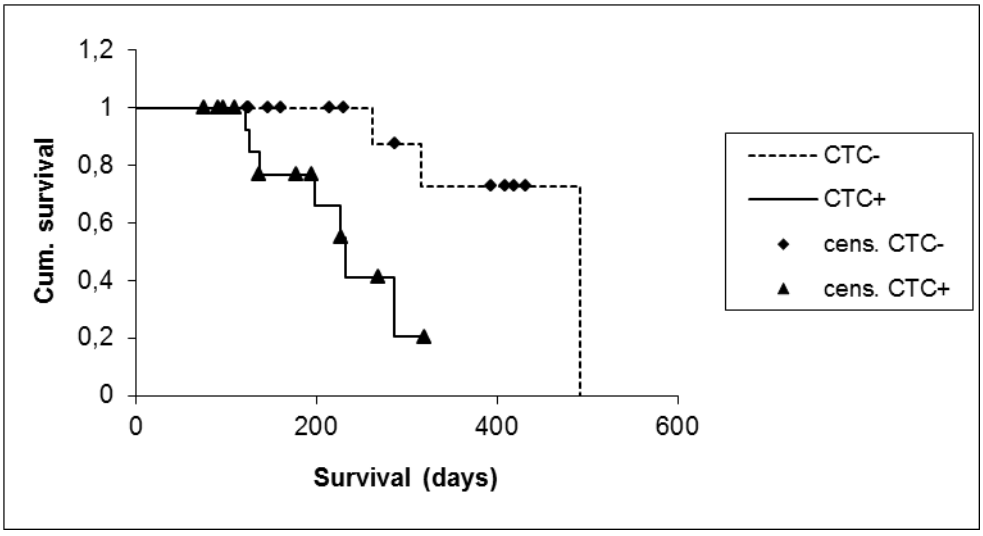

Figure 1. Survival curves for *AdnaTest* positive (CTC+) and *AdnaTest* negative (CTC–) cohorts.

Clinical studies

- Tewes, M. et al. (2009) Molecular profiling and predictive value of circulating tumor cells in patients with metastatic breast cancer: an option for monitoring response to breast cancer related therapies. Breast Cancer Res Treat. 2009 Jun;**115(3)**:581–90. doi: 10.1007/s10549-008-0143-x. Epub 2008 Aug 5.

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