A new blood test, ADtect®, has been developed that can aid the early detection of Alzheimer’s Disease (AD). The test is based on measuring the expression of selected genes in blood and is defined as the AD-specific gene signature. ADtect® comprises a low density array of 96 selected gene assays using RNA extracted from a blood sample. The performance of each of the 96 gene assays is calculated with an algorithm resulting in a positive or negative test score value indicating the presence or absence of AD.

Introduction
Although there is a lack of treatment options to arrest the more debilitating symptoms of Alzheimer’s disease (AD), early diagnosis and active management strategies can temporarily delay the onset. Diagnosis of AD today involves detailed clinical interviews, cognitive tests and imaging techniques. Despite the variety of testing approaches, there is still variability in diagnostic accuracy and it remains difficult to make an accurate diagnosis at an early stage of disease.

The DiAgentic ADtect® Test: Basic Principles
• The primary part of the disease is not the only part responding to a disease.
• The disease also leaves a unique “signature” that includes subtle systemic changes in gene expression, in other parts of the body.
• This signature can be identified using gene expression technology in peripheral blood.

Several independent papers have recently indicated that a peripheral blood based test could be used for diagnostic profiling in neurological diseases1-6. The development of ADtect® test (see Box 2) was based on whole genome screening of more than 32000 genes. During these development studies it was clearly shown that AD patients could be identified with a blood-based gene expression test7-9. Moreover, AD could be accurately distinguished from another neurodegenerative disease such as Parkinson’s disease10.

Development of ADtect®

The ADtect® gene expression based test has now been designed on a commercially available real-time PCR platform that utilizes micro fluidic cards (MFC). An outline of the test procedure is shown below in Box 3.

The ADtect® test procedure
Sample Preparation
Whole blood is collected from individuals in PaxGene™ Blood RNA tubes and processed according to manufacturers instructions. Total RNA is extracted from blood samples using PaxGene™ Blood RNA kit and quality assessed by NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer. Samples are prepared using the high-capacity cDNA reverse transcriptase kit and the Universal PCR Master Mix reagent from Applied Biosystems. Up to 4 individual samples are applied to the ADtect® MFC.

Gene sets and expression analysis
The gene expression analysis is done on the ABI Prism 7900HT Fast System using an ADtect® MFC with a 96-assay format, containing an AD-specific gene signature in a custom format, such that 4 individual samples can be run in parallel on each MFC (see Box 3). The gene assays were selected based on the performance characteristics from previous studies using an Applied Biosystems Whole Genome Array and TaqMan® MFC (see development phases outlined in Box 2). The dedicated ADtect® software analyzes the real-time PCR data and performs the disease classification. Data parameters/identifiers for each gene parameter has been implemented in the final disease specific algorithm.

Clinical diagnosis
Clinical diagnosis was determined at each site based on clinical interviews, cognitive testing and neuroimaging data. A final diagnosis was established for each case by a panel of clinical AD experts.

Results
During calibration of the ADtect® test, the disease algorithm was developed using partial least square regression on a balanced set of samples from Alzheimer’s disease patients (N=105) and cognitively healthy controls (N=110) recruited from Norwegian and Swedish hospitals. Leave-one-out cross-validation showed a 72% agreement of the ADtect® test with the consensus approved clinical diagnosis. The disease algorithm was built into the ADtect® software for patient reporting. A separate validation of the ADtect® test was performed in a multi-centre study including 4 blood sample collection sites in Norway. All samples (N=174, 32 AD, 42 cognitively healthy controls) were independent from the calibration study, and 3 of the recruitment sites were independent from the validation. The performance of these samples was shown to achieve at least 71.6% agreement of the ADtect® test with the clinical diagnosis.

The combined results are presented in Box 4. Overall the ADtect® test showed 73.8% agreement with the clinical diagnosis. Some variation (67-82%) was observed between sites, which could be attributed to uncertainty in the clinical evaluations and/or differences in sample handling. The test also showed similar performance characteristics in both mild and moderate AD cases (see MMSE subgroup table in Box 4).

There were no significant effects observed for the most common co-morbidities, such as diabetes, coronary disease, depression, previous strokes, hypertension, cancer and rheumatoid arthritis.

Discussion
The gene assays included in the ADtect® test cover a wide range of known functions associated with AD pathology, such as Abeta, pTau, presenilin and mitochondrial processing. A representative selection of these are shown in Box 6. The function and sphere of influence of these genes clearly support the clinical findings observed for the ADtect® test.

These genes appear to play a role in the biology and pathogenesis may also help in the further development of the test.

Conclusions
• A novel blood test has been developed that can aid the early detection of Alzheimer’s Disease using a peripheral blood sample.
• The ADtect® gene signature includes genes with key biological functions reported to be implicated in Alzheimer’s disease pathophysiology.
• The test has been developed in cooperation with the In Vitro Diagnostic Medical Device Directive 98/79/EC.
• The test is a relevant biomarker for early detection of Alzheimer’s disease.

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