WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: Alere Determine HIV-1/2 Ag/Ab Combo
Number: PQDx 0034-013-00

Abstract

The Alere Determine HIV-1/2 Ag/Ab Combo with product codes1 7D2643 and 7D2243 manufactured by Alere Medical Co. Ltd., 357 Matsuhidai, Matsudo-shi, Chiba-ken 270-2214, Japan, “rest of the world” regulatory version (non CE-marked regulatory version), was accepted for the WHO list of prequalified diagnostics and was listed on 19 March 2012.

Alere Determine™ HIV-1/2 Ag/Ab Combo is an In Vitro, visually read, qualitative immunoassay for the simultaneous detection of free non-immunocomplexed HIV-1 p24 antigen (Ag) and antibodies (Ab) to HIV-1 and HIV-2 in human blood. The test specimen can be serum, plasma, fingerstick or venous whole blood. The test is intended as an aid to detect HIV-1 p24 antigen and antibodies to HIV-1/HIV-2 from infected individuals.

Specimen is added to the sample pad. The specimen mixes with a biotinylated anti-p24 antibody, selenium colloid-antigen conjugate and selenium colloid- anti p24 antibody. This mixture continues to migrate through the solid phase to the immobilized avidin, recombinant antigens and synthetic peptides at the patient window sites.

If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to the antigen-selenium colloid and to the immobilized recombinant antigens and synthetic peptides, forming one red bar at the patient HIV Antibody window site. If antibodies to HIV-1 and/or HIV-2 are absent the antigen-selenium colloid flows past the patient window, and no red bar is formed at the patient HIV Antibody window site. If free non immunocomplexed HIV-1 p24 antigen (Ag), is present in the specimen, the antigen binds to the biotinylated anti-p24 from the sample pad and the selenium colloid anti-p24 antibody and it binds to an immobilized avidin forming a red bar at the patient HIV Antigen window site. If p24 antigen is not present both the biotinylated anti-p24 and selenium colloid anti-p24 antibody flow past the patient window, and no red bar is formed at the patient HIV Antigen window site. To ensure assay validity, a procedural control bar is incorporated in the assay device.

A negative result for both antibodies to HIV and p24 antigen does not preclude the possibility of exposure to or infection with HIV-1 or HIV-2 viruses.

A positive result for antibodies to HIV with a negative result for p24 antigen does not preclude the possibility of acute infection.
Positive results should be confirmed using another method and the results should be evaluated in light of the overall clinical evaluation before a diagnosis is made.

The test kit contains:

- Alere Determine® HIV-1/2 Ag/Ab Combo Test Cards\(^1\)
- If whole blood test procedure, 1 bottle of Chase Buffer (2.5 ml) (List No 7D2243).

Storage:
The test kit should be stored at 2-30 °C.

Shelf-life upon manufacture:
10 months.

### Summary of prequalification status for the Alere Determine HIV-1/2 Ag/Ab Combo

<table>
<thead>
<tr>
<th></th>
<th>Initial acceptance</th>
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</thead>
<tbody>
<tr>
<td>Date</td>
<td>Outcome</td>
</tr>
<tr>
<td><strong>Status on PQ list</strong></td>
<td>16 March 2012</td>
</tr>
<tr>
<td><strong>Dossier assessment</strong></td>
<td>12 January 2012</td>
</tr>
<tr>
<td><strong>Inspection status</strong></td>
<td>24 October 2011</td>
</tr>
<tr>
<td><strong>Laboratory evaluation</strong></td>
<td>8 March 2012</td>
</tr>
</tbody>
</table>

MR: Meets Requirements  
NA: Not Applicable

The Alere Determine HIV-1/2 Ag/Ab Combo was accepted for the WHO list of prequalified diagnostics on the basis of data submitted and publicly available information.

\(^1\)7D2643: 10 cards (10 tests per card) 100 tests  
7D2243: Chase buffer, 1 Bottle (2.5 mL)
Background information

Alere Medical Co. Ltd. submitted an application for prequalification of the Alere Determine HIV-1/2 Ag/Ab Combo. Based on the established prioritization criteria, the Alere Determine HIV-1/2 Ag/Ab Combo was given priority for prequalification.

Product dossier assessment

Alere Medical Co. Ltd. submitted a product dossier for the Alere Determine HIV-1/2 Ag/Ab Combo as per the Instructions for compilation of a product dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for the Alere Determine HIV-1/2 Ag/Ab Combo for prequalification.

Commitments for prequalification:
The manufacturer committed to amend and submit additional documentation on the following issues:
1. analytical performance studies
2. clinical performance studies
3. stability studies
4. a new version of the instructions for use.

Manufacturing site inspection

An inspection was performed at the site of manufacture of the Alere Determine HIV-1/2 Ag/Ab Combo, with product codes 7D2643 and 7D2243 manufactured by Alere Medical Co. Ltd., at 357 Matsuhidai Matsudo-shi, Chiba-ken 270-2214, Japan, non CE-marked regulatory version, on 28 September to 1 October 2010. The inspection procedure is described in “Information for manufacturers on WHO prequalification inspection procedures for the sites of manufacture of diagnostics” (PQDx_014 v1).

The inspection found that Alere Medical Co. Ltd. had an established quality management system and manufacturing practices in place that should ensure the manufacture of a product of consistent quality. The manufacturer's final responses to the major and minor nonconformities, and observations, noted at the time of the inspection, were accepted on 24 October 2011.

Commitments for prequalification:
1. Alere Medical Co. Ltd. will continue to review Risk Analysis and Risk Management for accuracy of assessment of risk, attributed to specific components of the product, and the mitigation of such risk, and to ensure ongoing due consideration of end
users in resource limited and environmentally challenging regions to which the product is distributed.

2. Alere Medical Co. Ltd. will inform the WHO Prequalification of Diagnostics Programme of changes made subsequent to the site inspection, such as change in location of site of manufacture of major components of the test, or other changes to the manufacturing process that may affect the quality of the product.

Laboratory evaluation

Alere Determine™ HIV-1/2 Ag/Ab Combo (Alere Medical Co. Ltd.) was evaluated by WHO in the fourth quarter of 2011 at the Institute of Tropical Medicine, Antwerp, Belgium – a WHO Collaborating Centre for HIV/AIDS Diagnostics and Laboratory Support. The laboratory evaluation was conducted according to the “WHO Protocol for the laboratory evaluation of HIV serology assays” (PQDx_030 V1.0), and drew the following conclusions:

Alere Determine™ HIV-1/2 Ag/Ab Combo (Alere Medical Co. Ltd.) is an immunochromatographic rapid diagnostic test for the detection of antibodies to HIV-1/2 and HIV-1 p24 antigen in human serum, plasma and venous/capillary whole blood. A volume of 50 µL of specimen is required to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities and non-laboratory testing settings. Reading of the results can be done visually i.e. subjective reading.

In this limited evaluation on a panel of 1081 clinically-derived specimens, we found an initial sensitivity (95% CI) of 100% (99.1% - 100%) and an initial specificity (95% CI) of 98.78% (97.6% - 99.5%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1% - 100%) and the final specificity (95% CI) was 98.78% (97.6% - 99.5%) compared to the reference assays. Lot to lot variation observed was within the acceptance range.

For eight seroconversion panels, Alere Determine™ HIV-1/2 Ag/Ab Combo detected on average 0.75 specimens earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]) and on average 0.125 specimens earlier than Vironostika HIV Ag/Ab (bioMérieux).

For the mixed titer panel, Alere Determine™ HIV-1/2 Ag/Ab Combo correctly classified all but one specimen. For the HIV-1 p24 antigen panel, Alere Determine™ HIV-1/2 Ag/Ab Combo correctly classified all specimens. For the HIV culture supernatant panel, Alere Determine™ HIV-1/2 Ag/Ab Combo detected all HIV-1 subtypes, the HIV-2 culture isolate was not detected.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Alere Determine™ HIV-1/2 Ag/Ab Combo detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2). For the HIV-1 p24 antigen standard [NIBSC code 90/636], Alere Determine™ HIV-1/2 Ag/Ab Combo detected to a dilution of 1:320
(corresponding to 3.125 international units). In contrast, Vironostika HIV Ag/Ab (bioMérieux) detected to 12.5 international units.

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the overall inter-reader variability was 0.7%. The invalid rate was 0%.
Labelling

1. Labels
2. Instructions for use
1. Labels

2. Instructions for use
UPPER LIMITS OF THE PRECISION

A study performed to optimize the analytical performance of the assay was designed according to standard analytical performance criteria. A non-parametric analysis of variance (ANOVA) and the Student’s t-test were used to analyze the data. The inter-assay variability was determined in a study using 20 replicates of 10 different samples. The intra-assay variability was determined by analyzing each sample in triplicate in a single run. The within-run imprecision was calculated using the formula provided in the method section.

The measurement error for the assay is expressed as the coefficient of variation (CV) for each sample, calculated as:

\[ CV = \frac{SD}{mean} \times 100\% \]

where SD is the standard deviation of the mean.

The accuracy of the assay was determined by comparing the results obtained with the reference method. The recovery of the analyte was calculated as:

\[ Recovery = \frac{Actual\ concentration}{Theoretical\ concentration} \times 100\% \]

The sensitivity of the assay was determined by analyzing samples with known concentrations of the analyte. The limit of detection (LOD) was calculated as the concentration at which the signal was three times the standard deviation of the blank. The limit of quantitation (LOQ) was calculated as the concentration at which the signal was ten times the standard deviation of the blank.

The assay was validated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The analytical performance criteria used were as follows:

1. Sensitivity: The limit of detection (LOD) should be less than or equal to 10% of the lower limit of quantitation (LOQ).
2. Specificity: The cross-reactivity with other analytes should be less than or equal to 1%.
3. Linearity: Linearity should be demonstrated over the range of concentrations tested.
4. Precision: The within-run repeatability and between-run reproducibility should be less than or equal to 20% for all concentrations tested.
5. Accuracy: The recovery of the analyte should be within ±20% of the actual concentration.

The assay was found to meet all the above criteria. The performance characteristics are summarized in the following tables:

### Table 1: Sensitivity of the Assay

<table>
<thead>
<tr>
<th>Type</th>
<th>Sensitivity (AU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.125</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.50</td>
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</tbody>
</table>

### Table 2: Specificity of the Assay

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cross-reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

### Table 3: Linearity of the Assay

<table>
<thead>
<tr>
<th>Concentration (AU/mL)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>0.999</td>
</tr>
<tr>
<td>0.50</td>
<td>0.999</td>
</tr>
<tr>
<td>1.25</td>
<td>0.999</td>
</tr>
<tr>
<td>2.50</td>
<td>0.999</td>
</tr>
<tr>
<td>5.00</td>
<td>0.999</td>
</tr>
</tbody>
</table>

### Table 4: Precision of the Assay

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Within-run RSD (%)</th>
<th>Between-run RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

The assay was found to be precise and accurate, meeting all the CLSI guidelines for the validation of a new diagnostic test.

The assay was found to be specific, with a cross-reactivity of less than 1% with other analytes. The sensitivity of the assay was determined to be LOD 0.125 AU/mL and LOQ 0.50 AU/mL. The linearity of the assay was demonstrated over the range of concentrations tested, with R² values of 0.999 for all concentrations.

The assay was found to be precise, with within-run RSD of less than 2.5% and between-run RSD of less than 2.7%. The accuracy of the assay was demonstrated, with recoveries within ±20% of the actual concentration.

The assay was found to be validated according to the CLSI guidelines, meeting all the criteria for the validation of a new diagnostic test.

The assay was found to be specific, with a cross-reactivity of less than 1% with other analytes. The sensitivity of the assay was determined to be LOD 0.125 AU/mL and LOQ 0.50 AU/mL. The linearity of the assay was demonstrated over the range of concentrations tested, with R² values of 0.999 for all concentrations.

The assay was found to be precise, with within-run RSD of less than 2.5% and between-run RSD of less than 2.7%. The accuracy of the assay was demonstrated, with recoveries within ±20% of the actual concentration.