This package insert must be read carefully prior to use of this product.

**Dermatophyte Test Strip**

**Diafactory Tinea Unguium**

For 10 tests

**[General Precautions]**

1. Diafactory Tinea Unguium (this kit) is only intended for in vitro diagnostic use, and must not be used for any other purposes.

2. The user should evaluate the result of this assay comprehensively in conjunction with other test results and the clinical symptoms.

3. This kit should only be used as directed. The reliability of values cannot be guaranteed if this kit is used for other purposes or if tests are conducted by other methods than stated in this manual.

**[Description (Kit Components)]**

<table>
<thead>
<tr>
<th>Components</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Test Strips</td>
<td>Anti-Dermatophyte Antibody Anti-Dermatophyte Antibody with Gold Colloid</td>
</tr>
<tr>
<td>2 Extraction Buffers</td>
<td>Buffer</td>
</tr>
</tbody>
</table>

**[Intended Use]**

Detection of Dermatophyte-derived antigens in nails (as a support of a diagnosis of tinea unguium).

**[Principle of the Test]**

Diafactory Tinea Unguium is a lateral flow immunoassay intended to detect Dermatophyte-derived antigens in nails using anti-Dermatophyte antibody that has been immobilized on a nitrocellulose membrane. The test strip used in this kit is composed of a sample pad, a reagent pad, a test paper and an absorbent pad (Figure 1). The test line zone indicates a Dermatophyte antibody with gold colloid in the dry state, and the test paper contains anti-Dermatophyte antibody in the dry state affixed on the test line zone and the dye in the dry state affixed on the control line zone. This dye is a colorless dye at a pH of 6.5 or higher and a pink dye at a pH of 7.0 or higher, and allows the user to confirm that a specimen has correctly passed through the test line zone.

**[Assay Procedure]**

1. **Preparation**
   - (1) Prepare the required quantities of test strips, test tubes and extraction buffer.
   - (2) Add 0.25 to 0.5 mL of the extraction buffer to the test tube (Figure 2). Put the specimen in the test tube and place it in a test tube rack for at least 1 minute.

2. **Collection and Preparation**
   - (1) Take a specimen from deep inside the nail plate.

3. **Interpretation of Results**
   - (1) If a pink band appears in the control line zone and a purple band appears in the test line zone, the test is positive.
   - (2) If no pink band appears in the control line zone after 5 to 30 minutes, the test is invalid.
   - (3) If a band appears in the test line zone after 30 minutes or longer, it indicates a negative result.

4. **Other Precautions**
   - (1) The result may be judged to be positive if colored bands are found both on the test line zone and the control line zone after at least 5 minutes have elapsed. Similarly, this result may be judged to be negative if no visible band appears on the test line zone and a pink band appears on the control line zone after at least 5 minutes have elapsed.

**[Clinical Significance]**

Diafactory Tinea Unguium, unlike direct microscopy, does not require special equipment to determine whether Dermatophyte is present or absent, and this kit, unlike PCR, does not require special equipment. Diafactory Tinea Unguium, which is easy to use and provides quick results, is an effective assay for the rapid diagnosis of tinea unguium.

**[Performance]**

1. **Sensitivity and Accuracy**
   - When a weak control specimen was tested, this kit provided a negative result.
   - When a positive control specimen and a positive control specimen were tested, this kit provided positive results.

2. **Within-run reproducibility**
   - When a negative control specimen was tested 4 times, the kit provided a negative result every time.
   - When a weakly positive specimen and a positive control specimen, respectively, were tested 4 times, the kit provided a positive result every time.

3. **Minimum Detectable Sensitivity**
   - Trochophos ruber (NBRC 9185), 0.5 μg dry weight/mL.

4. **Reference Standard for Calibration**
   - Dry cells of Trochophos ruber (NBRC 9185)

5. **Cross-reactivity**
   - Autoclaved dry cells of various other fungi than Dermatophyte were added to the extraction buffer at a concentration of 300 μg/mL to evaluate the influence of each fungus on the assay. In addition, colonies of various bacteria grown on agar plates were added to the extraction buffer to evaluate the influence of each bacterium on the assay. This test was not reactive with the tested fungi (non-Dermatophyte) shown below:

   - Aspergillus nidulans, Penicillium citrinum, Scopulariopsis brevicaulis, Alternaria alternata, Paecilomyces variotii, Scopulariopsis squaligena, Penicillium waksmanii, Scopulariopsis commune (1 nucleus), Scopulariopsis commune (2nuclei), Absidia coerulea, Buddelloa ranarum, Cunninghamella bertholletiae, Mortierella subfulva, Mucor circinelloides, M. rouxii, Rhizopus oryzae, Rhizopus stolonifer var. reflexus, Syncphalastrum racemosum,

   - **[Table 1]**

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Concentration (μg/mL)</th>
<th>Influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbinafine</td>
<td>0.5</td>
<td>Not observed</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>100</td>
<td>Not observed</td>
</tr>
</tbody>
</table>

   - **[Figure 2]**

   - (3) It is desirable that the specimen amount should be 1 μg or more.

   - **[Figure 3]**

   - (3) The influence of antifungals (1, terbinafine, itraconazole) are commonly used for the treatment of tinea unguium on this assay were evaluated. When the negative control specimen, the positive control specimen and the antifungal reactive species which was prepared by diluting the positive control specimen with the antifungal and subjected to this kit, no influences of these drugs were observed. The concentration of each drug added was approximately 100 times the MIC (minimum inhibitory concentration).

   - **[Figure 4]**

   - (4) If a pink band appears in the control line zone and a purple band appears in the test line zone, the test is positive. If no pink band appears in the control line zone after 5 to 30 minutes, the test is invalid.

   - **[Figure 5]**

   - (5) Let the test strip stand for at least 5 minutes and determine the result (positive, negative or invalid) by visually checking the presence or absence of colored bands in the control line zone and the test line zone, within 30 minutes after standing the test strip in the test tube.

   - **[Figure 6]**

   - (6) Read the result within 30 minutes. The result may be judged to be positive if colored bands are found both on the test line zone and the control line zone after at least 5 minutes have elapsed. Similarly, this result may be judged to be negative if no visible band appears on the test line zone and a pink band appears on the control line zone after at least 5 minutes have elapsed.

   - **[Figure 7]**

   - (7) If a band appears in the test line zone after 30 minutes or longer, it indicates a negative result.
In 222 patients (at 11 centers) suspected of having tinea unguium on visual inspection, a specimen was collected from a foot or hand nail according to the guidelines for diagnosis and treatment of cutaneous fungal infection. The specimens were crushed into 3 pieces and subjected to measurement with this kit, direct microscopy and PCR (only specimens for which the results of this kit and direct microscopy were inconsistent), respectively. Specimen collection, direct microscopy, this kit and PCR were performed by different persons under blinded conditions.

2. Results of Clinical Performance Study

In 222 patients (at 11 centers) suspected of having tinea unguium on visual inspection, a specimen was collected from a foot or hand nail according to the guidelines for diagnosis and treatment of cutaneous fungal infection. The specimens were crushed into 3 pieces and subjected to measurement with this kit, direct microscopy and PCR (only specimens for which the results of this kit and direct microscopy were inconsistent), respectively. Specimen collection, direct microscopy, this kit and PCR were performed by different persons under blinded conditions.

(1) Comparison between the results of Diafactory Tinea Unguim and PCR incorporating direct microscopy

Analyses were performed on 222 patients. In 5 patients in whom the results of this kit and direct microscopy were inconsistent and PCR could not be performed because the amount of specimen was insufficient, the result of direct microscopy was used.

Table 2. Comparison between the Results of Diafactory Tinea Unguim and PCR incorporating Direct Microscopy

<table>
<thead>
<tr>
<th>Result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diafactory Tinea Unguim</td>
<td>196</td>
<td>8</td>
<td>204</td>
</tr>
<tr>
<td>PCR incorporating direct microscopy</td>
<td>202</td>
<td>28</td>
<td>230</td>
</tr>
</tbody>
</table>

Sensitivity, 97.0% Specificity, 75.0% Accuracy, 93.5% Negative predictive value, 71.4% Positive predictive value, 97.5%

(2) Comparison between the results of Diafactory Tinea Unguim and the dermatologist’s final diagnosis (based on the results of direct microscopy, PCR, clinical manifestation, specimen collection site, etc.)

Analyses were performed on 217 patients, excluding 5 patients in whom PCR could not be performed because the amount of specimen was insufficient and a final diagnosis could not be made.

Table 3. Comparison between the Results of Diafactory Tinea Unguim and the Final Diagnosis

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>Tinea Unguim</th>
<th>Not tinea Unguim</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diafactory Tinea Unguim</td>
<td>196</td>
<td>2</td>
<td>198</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>17</td>
<td>217</td>
</tr>
</tbody>
</table>

Sensitivity, 98.0% Specificity, 88.2% Accuracy, 97.2% Negative predictive value, 78.9% Positive predictive value, 99.0%

[Precautions for Use and Handling]

1. Precautions for Handling (to Prevent Danger)

(1) If the extraction buffer comes in contact with the eyes, mouth, or skin, rinse thoroughly with running water as first aid, and seek medical treatment if necessary.

(2) When handling specimens and this kit, wear a mask, gloves and other protective apparel. Wash hands thoroughly after testing.

(3) All specimens used for the test should be handled as if potentially infectious. Used test strips, extracted samples, test tubes and stir rods should be handled in the same manner as specimens.

(4) To prevent infections from spilled specimens or solutions containing specimens, wipe the spilled and contaminated area thoroughly with disinfectant such as sodium hypochlorite solution.

2. Precautions for Use

(1) The reagents in this kit can be used only for the detection of Dermatophyte-derived antigens in nails.

(2) Use clean instruments when taking specimens.

(3) Do not use the kit beyond the expiration date.

(4) Each test strip, extraction buffer, test tube and stir rod in the kit can only be used once. Do not re-use them.

(5) This kit should be stored at 2 to 30°C. Avoid freezing and exposure to direct sunlight.

(6) Do not combine reagents of different lots.

3. Precautions on Disposal

(1) Before disposal, use test strips and containers must be autoclaved at 121°C for 20 minutes or soaked in a sodium hypochlorite solution for longer than 3 hours, as if potentially infectious.

(2) Used containers etc. must be either incinerated or disposed of as medical or industrial waste according to the applicable waste disposal regulations.

[Storage and Shelf Life]

- Shelf life: 36 months from the date of manufacture (The expiration date is printed on the outer package.)

[Packaging Contents]

DE001 ——— 10 test kit

[References]
