WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: AiD™ anti-HIV 1+2 ELISA
Number: PQDx 0006-005-00

Abstract

AiD™ anti-HIV 1+2 ELISA with product codes WI-4396 and WI-43480, manufactured by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, rest of world regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 15 February 2016.

Intended use:
AiD™ anti-HIV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M - O) or type 2 in human serum or plasma samples. The assay can be utilized for screening of blood donors and/or as an aid in the diagnosis of clinical conditions related to infection with HIV-1 and/or HIV-2, the etiological agents of the acquired immunodeficiency syndrome (AIDS).

Assay description:
AiD™ anti-HIV 1+2 ELISA is a two-step incubation antigen “sandwich” enzyme immunoassay, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens expressed in E.coli (recombinant HIV-1gp41, gp120, and recombinant HIV-2 gp-36). Patient specimen (serum or plasma) is added, and during the first incubation step, the specific HIV1/2 antibodies will be captured inside the wells, if present. The microwells are then washed to remove unbound proteins. A second set of recombinant antigens conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigens is added, and during the second incubation, they will bind to the captured antibody. The microwells are washed to remove unbound conjugate, and Chromogen solutions are added into the wells. In wells containing the antigen-antibody-antigen(HRP) “sandwich” immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after the reaction is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing specimens negative for anti-HIV 1/2 remain colorless.

Reactive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
### Test kit contents:

<table>
<thead>
<tr>
<th></th>
<th>96 tests (product code WI-4396)</th>
<th>480 tests (product code WI-43480)</th>
</tr>
</thead>
</table>
| **Microwell Plate:**     | Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. Each well contains recombinant HIV 1/2 antigens (recombinant HIV-1 gp41, gp120, and recombinant HIV-2 gp36). The microwell strips can be broken to be used separately. | 1x 96 wells  
12x 8 well plate  
5x 96 wells  
12x 8 configuration |
| **Negative Control:**    | Protein-stabilized buffer tested non-reactive for HBsAg, and antibodies to HIV 1/2, HCV and TP Yellow-colored liquid filled in a vial with green screw cap. Preservative 0.1% ProClin™ 300. | 1x 1ml vial  
3x 1ml vial |
| **Positive Control-1:**  | Red-colored liquid filled in a vial with red screw cap. Protein-stabilized buffer solution tested positive for antibodies to HIV-1. Preservative 0.1% ProClin™ 300. | 1x 1ml vial  
3x 1ml vial |
| **Positive Control-2:**  | Protein-stabilized buffer solution tested positive for antibodies to HIV-2. Red-colored liquid filled in a vial with yellow screw cap. Preservative 0.1% ProClin™ 300. | 1x 1ml vial  
3x 1ml vial |
| **Hrp-Conjugate:**       | Horseradish peroxidase-conjugated recombinant HIV 1+2 antigens. Red-colored liquid in a white vial with red screw cap. Preservative 0.1% ProClin™ 300. | 1x 12ml vial  
5x 12ml vial |
| **Wash Buffer:**         | Detergent Tween-20. Colorless liquid filled in a clear bottle with white screw cap. Buffer solution containing detergent. The concentrate must be diluted 1 to 20 with distilled/ deionized water before use. DILUTE BEFORE USE! | 1x 50ml bottle  
2x 125ml bottle |
| **Chromogen Solution A:**| Urea peroxide solution. Colorless liquid filled in a white vial with green screw cap. | 1x 8ml vial  
1x 60ml vial |
| **Chromogen Solution B:**| Tetramethyl benzidine. Colorless liquid filled in a black vial with black screw cap. | 1x 8ml vial  
1x 60ml vial |
| **Stop Solution:**       | Diluted sulfuric acid solution (0.5M H$_2$SO$_4$). Colorless liquid in a white vial | 1x8ml vial  
1x60ml vial |
with yellow screw cap.

**Plastic Sealable Bag:** For enclosing the strips not in use.

**Instructions For Use**

**Cardboard Plate Cover**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Plastic Sealable Bag</td>
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<td>5</td>
</tr>
<tr>
<td>Instructions For Use</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cardboard Plate Cover</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

**Storage:**
The test kit should be stored at 2 to 8 °C.

**Shelf-life:**
15 months.

### Summary of prequalification status for AiD anti-HIV 1+2 ELISA

<table>
<thead>
<tr>
<th></th>
<th>Date</th>
<th>Outcome</th>
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<tr>
<td>Status on PQ list</td>
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<td>listed</td>
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<tr>
<td>Dossier assessment</td>
<td>19 July 2013</td>
<td>MR</td>
</tr>
<tr>
<td>Inspection status</td>
<td>24 April 2015</td>
<td>MR</td>
</tr>
<tr>
<td>Laboratory evaluation</td>
<td>15 January 2014</td>
<td>MR</td>
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</table>

MR: Meets Requirements  
NA: Not Applicable

AiD™ anti-HIV 1+2 ELISA was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

### Background information

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd submitted an application for prequalification of AiD™ anti-HIV 1+2 ELISA. Based on the established prioritization criteria, AiD™ anti-HIV 1+2 ELISA was given priority for prequalification.

### Product dossier assessment

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd submitted a product dossier for AiD™ anti-HIV 1+2 ELISA 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 AiD™ anti-HIV 1+2 ELISA for prequalification.
Commitments for prequalification: N/A

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (No. 31 Life Science Park Road Changping District, Beijing, China) of AiD™ anti-HIV 1+2 ELISA in December 2014 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality. The manufacturer's responses to the nonconformities to the quality management system found at the time of the inspection were accepted 24 April 2015.

Commitments for prequalification: N/A

Laboratory evaluation

AiD™ anti-HIV 1+2 ELISA (Beijing Wantai Biological Pharmacy Enterprise, Co. Ltd.) was evaluated by WHO in the fourth quarter of 2012 using serum/plasma specimens. From this evaluation, we drew the following conclusions:

AiD™ anti-HIV 1+2 ELISA (Beijing Wantai Biological Pharmacy Enterprise, CO, Ltd.) is an enzyme immunoassay assay for the detection of HIV-1/2 antibodies in human serum/plasma. A volume of 100 μL of specimen is needed to perform the assay. This type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. Reading of the results must be performed with a spectrophotometer.

In this limited evaluation on a panel of 1118 clinically-derived specimens, we found an initial sensitivity (95% CI) of 100% (99.2% - 100%) and an initial specificity (95% CI) of 99.09% (98.0% - 99.7%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.2% - 100%) and the final specificity (95% CI) was 99.54% (98.7% - 99.9%) compared to the reference assays. Lot to lot variation was acceptable.

For eight seroconversion panels, AiD™ anti-HIV 1+2 ELISA detected on average the same number of specimens as the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, AiD™ anti-HIV 1+2 ELISA correctly classified 24 out of the 25 specimens. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], AiD™ anti-HIV 1+2 ELISA correctly classified all specimens. In this study, 0% of the results were recorded as indeterminate. The invalid rate was 0%.
Labelling

1. Labels
2. Instructions for use
1. Labels

**Shipping box label**

![Image showing shipping box label]

**Kit box label for product code WI-4396**

![Image showing kit box label]

**Box label for product code WI-4396**

![Image showing box label]
### Box label for product code WI-43480

#### AID™ anti-HIV 1+2 ELISA

Enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) type 1 and/or type 2 in human serum or plasma.

<table>
<thead>
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<th>Components</th>
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<tr>
<td>UUU PLATE</td>
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</tr>
<tr>
<td>CONTROL 1</td>
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<tr>
<td>CONTROL 2</td>
<td>7</td>
<td>3x 1 ml</td>
</tr>
<tr>
<td>CONTROL 3</td>
<td>8</td>
<td>3x 1 ml</td>
</tr>
<tr>
<td>WASH BUF BOX</td>
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<td>1x 120 ml</td>
</tr>
<tr>
<td>CHROM SOL A</td>
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<td>1x 80 ml</td>
</tr>
<tr>
<td>CHROM SOL B</td>
<td>11</td>
<td>1x 80 ml</td>
</tr>
<tr>
<td>STOP SOL</td>
<td>12</td>
<td>1x 80 ml</td>
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</tbody>
</table>

#### Box label for product code WI-43480

![Box label for product code WI-43480](image-url)
Component labels for product code WI-4396

Component labels for Product code WI-43480
Read the page instructive and completely before performing the assay. Follow the instructions and do not modify them. Only by strictly adhering to the instructions, the erroneous results can be avoided and the optimal performance of AD² anti-HIV 1+2 ELISA achieved.

INCREASED USE

AD² anti-HIV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M-1) or type 2 in human serum or plasma specimens. The assay can be utilized for screening of blood donors and/or in the diagnosis of clinical conditions related to infection with HIV-1 and/or HIV-2, the etiological agents of the acquired immunodeficiency syndrome (AIDS).

SUMMARY

Sensitiveness of infection with HIV may be obtained by testing for the presence of HIV antibodies or antigens in individuals suspected for HIV infection. Antigen can generally be detected during both acute phase and the symptomatic phase of AIDS. The antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the whole infection period, starting at or shortly after the acute phase and lasting till the end stage of AIDS[9]. Therefore, the use of highly sensitive antibody assays is the primary approach in serodiagnosis of HIV infection. Apart from sexual transmission, the principal route of infection with HIV is blood transfusion. HIV can present both in cellular and cell-free fractions of human blood. Sensitivity of the ELISA test is dependent on the number of HIV transmission through contaminated blood. This can be effectively achieved by testing for the antibodies to HIV-1 and HIV-2 using a highly specific AD² ELISA[10].

P R I N C I P L E 0 F T H E T E S T

AD² anti-HIV 1+2 ELISA is a two-step incubation antigen "sandwich" enzyme immunoassay kit, which uses polyclonal antibodies to the viral envelope antigens gp36 and gp41 (HIV-1(DO) and recombinant HIV-2(gp36)). Patient's serum or plasma specimen is added, and during the first incubation step, the specific HIV-1/2 antibody will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. A second set of recombinant antigens conjugates to the enzyme Horseradish Peroxidase (HRP)-Conjugate (for HIV-1) and HRP-Conjugate (for HIV-2) during the second incubation, they will bind to the captured antibody. The microwells are washed to remove unbound conjugate, and Chromogen can then react with the remaining antibody-antigen/antibody complex, the colorless Chromogen is hydrolyzed by the bound HRP conjugate to a blue colored product. The absorbance of each microwell can be measured and it is proportional to the amount of antibody captured in the wells, and to the specimen respectively. Wells containing specimens non-reactive for an HIV-1/2 negative control.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label package when stored between 2-8°C. To assure maximum performance of AD² anti-HIV 1+2 ELISA, during storage, protect the reagents from contamination with micromaterials.

PRECAUTIONS AND SAFETY

BE USED TO ONLY END QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test steps procedure and do not modify them.

1. Do not exchange reagents from different kits or use reagents from other commercial available kits. The cross reactivity of the kits is precisely matched for optimal performance of the test.
2. Make sure that all reagents are within the validity indicated on the kit box and the same lot. Never reuse reagents. Reagents from different lots may cause inaccuracy or false results.
3. CAUTION - CRITICAL STEP. Allow the reagents and specimens to reach room temperature (15-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use. Use only one volume of specimen. Failure to do so, may cause inaccuracy or false readings at the assay.
4. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
5. Never allow the microplate wells to dry after washing in the washer. Proceed immediately to the next step.
6. Avoid the formation of air bubbles when adding the reagents.
7. Avoid the use of the same test vials for different working conditions for all kits.
8. Calibrate the plate reader frequently to assure the accuracy of specimens/diagram. Use different plate reader and make sure the plate reader that can guarantee to avoid cross-contamination.
9. Ensure that the incubation temperature at 37°C inside the incubator. Without proper incubation, the test results will not be accurate. In the preparation of the Negative Control of the kit, these materials have been tested with spiked kits with accepted performance and found negative for HIV/HBV and antibodies to other infectious agents. A proper test method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme care to avoid infecting laboratory workers. Buffer solution (spiked with 100 IU/mL of live HIV-1 and HIV-2) derived from a healthy volunteer.
10. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never plate solutions with mouth. Chemicals can be handled and disposed of in accordance with the current GPL (Good Laboratory Practices) and the local or national regulations.
11. The pipette and other liquid transfer instruments should be cleaned and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further use. Beyond 2 hours, the sodium hypochlorite should never be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results, rather than the number of plates concurrently processed. The results are calculated by relating each specimen absorbance to a cut-off value (OD 0.1). The OD 0.1 cut-off value is based on single filter plate reader, the results should be calculated by subtracting the blank well x A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the blank well x A from the print report values of specimens and controls.
Calculation of the Cut-off value (C.O.) = N + 0.12
(N = the mean absorbance value for three negative controls).

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- The value of the Blank well, which contains only Chromogen and Stop solution, is ≤ 0.05 at 450 nm.
- The A value of the Positive control must be ≥ 0.20 at 450 nm or 455 nm after blanking.
- The A values of the Negative control must be ≤ 0.10 at 450/595 or 450 at 455 nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A value does not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:
1. Quality Control
Blank well A value: A1 = 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)
Well No.: B1 = 0.020 0.012 0.016
Well No.: E1 = 0.01
Positive control A values after blanking: 2.421 2.369
All control values are within the stated quality control range
2. Calculation of N: (B1-E1) / 4 = 0.016
3. Calculation of the Cut-off: (C.O.) = 0.016 +0.12 = 0.138

INTERPRETATIONS OF THE RESULTS

Negative Results (A/C.O. < 1): specimens giving absorbance less than the Cut-off value are non-reactive for this assay, which indicates that no anti-HIV-1/2 antibodies have been detected with AD™ anti-HIV-1/2 ELISA, therefore the patient is probably not infected with HIV-1 and the blood unit does not contain antibodies to HIV-1/2 and could be transfused in cases where infectious diseases markers are also absent.

Positive Results (A/C.O. ≥ 1): specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that anti-HIV-1/2 antibodies have been detected using AD™ anti-HIV-1/2 ELISA. All initial reactive specimens should be retested in duplicate using AD™ anti-HIV-1/2 ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered reactive for antibodies to HIV-1/2 with AD™ anti-HIV-1/2 ELISA.

Borderline (A/C.O. = 0.9-1.0): specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any repeatedly reactive specimen is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

LIMITATIONS

1. Positive results can be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals (e.g., AIDS). Negative results obtained with AD™ anti-HIV-1/2 ELISA are only an indication that the specimen does not contain detectable level of anti-HIV-1/2 antibodies and any negative result should not be considered as confirmative evidence that the individual is not infected with HIV-1/2 or is not infected with HIV-1/2.
3. If antibody test and initial reactive specimen, the assay results are negative, such specimen should be considered as non-reactive (false positive) and interpreted as negative. As many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with bad, but not limited to, inadequate washing step. For more information regarding Wanta’s ELISA Troubleshooting, please refer to Wanta’s “ELISA & Troubleshooting Guide”.

PeliSpy Multi-Marker results:

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<th>Percentage</th>
<th>s6a Min</th>
<th>s6a Max</th>
<th>Percentage</th>
<th>s6b Min</th>
<th>s6b Max</th>
<th>Percentage</th>
<th>s6c Min</th>
<th>s6c Max</th>
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<td>80</td>
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Wanta’s QC specimen results:

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<thead>
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<th>n</th>
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<th>95%</th>
<th>s6a</th>
<th>Min</th>
<th>s6a</th>
<th>Max</th>
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<th>Max</th>
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<th>Min</th>
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</tr>
</tbody>
</table>

SYMBOLS:

In Vitro Diagnostic Medical Device
• +2°C ~ +8°C Storage Conditions

Use By
Content Sufficient For 20 Tests
Instructions For Use
Catalog Number
Manufacturer

Beijing Wataldi Biological Pharmacy Co., Ltd.
No.31 Kessanjue Road, Changping District, Beijing 102205, China
Tel: +86-10-59508888 Fax: +86-10-59739049
Website: www.yteld.com
Email: xbox@yteld.com

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:
Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not inter-changeable.

1. Microtubes: Code 1
2. Negative Control: Code 2
5. HRP-Enzyme: Code 5
6. Wash Buffer: Code 6
7. Chromogenic Solution A: Code 7
8. Chromogenic Solution B: Code 8
9. Stop Solution: Code 9

SUMMARY OF THE ASSAY PROCEDURE:
Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add specimens
Incubate
Wash
Stop
Incubate 100 mins
Read the absorbance

EXAMPLE SCHEME OF CONTROLS / SPECIMENS DISPENSING:

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen 1</td>
<td>Specimen 2</td>
<td>Specimen 3</td>
<td>Specimen 4</td>
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</tbody>
</table>

REFERENCES:


Evaluation study carried out in Almarn, the Netherlands, between April and November 2005, demonstrated the following performance characteristics of AD™ anti-HIV-1/2 ELISA. The specificity of the kit was 99.85% as determined on all negative specimens (547) that were tested. When examined on the unsolicited donors (random and first time donors), the specificity was 99.5% (95% CI 99.5%-100%).

AD™ anti-HIV-1/2 ELISA test results on unsolicited donors:

<table>
<thead>
<tr>
<th>Panel</th>
<th>Number tested</th>
<th>Positive (A/C.O. &gt; 1)</th>
<th>Negative (A/C.O. = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>radium donor serum</td>
<td>250</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>Random plasma donors</td>
<td>1400</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>First time donors</td>
<td>980</td>
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<td>0.07</td>
</tr>
<tr>
<td>Total</td>
<td>3524</td>
<td>2</td>
<td>0.07</td>
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