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INTENDED USE

PF4 IgG is a qualitative screening assay for the detection of heparin associated IgG antibodies in human serum.

SUMMARY AND EXPLANATION

Patients receiving heparin treatment for at least a week often develop thrombocytopenia.\textsuperscript{1,2,3} In some cases the platelet levels are reduced only slightly and return to normal even when heparin treatment is continued. This type of thrombocytopenia is termed “Type I” heparin-induced thrombocytopenia (HIT) and is not antibody-mediated.\textsuperscript{2}

In other patients thrombocytopenia is usually more severe and is antibody-mediated. This condition is designated “Type II” HIT. Type I HIT is generally considered to be a benign condition, whereas patients with Type II HIT are at risk to develop more severe thrombocytopenia as well as arterial or venous thrombosis if heparin therapy is continued. Antibodies associated with Type II HIT can be detected in several ways. The most commonly used techniques are the platelet aggregation test,\textsuperscript{4} the serotonin release test\textsuperscript{5} and the platelet factor 4 ELISA.\textsuperscript{6,7,8}

It is now known that antibodies associated with Type II HIT recognize sites on a platelet protein designated “platelet factor 4” (PF4) that are created when PF4 is complexed with heparin or another linear polyanionic compound such as polyvinyl sulfonate (PVS).\textsuperscript{9,10,11}

PF4 IgG Solid Phase ELISA microwells provide immobilized PF4:PVS complexes as a target for the detection of IgG antibodies associated with Type II HIT.

PRINCIPLE OF THE PROCEDURE

Patient sample is added to microwells coated with platelet factor 4 (PF4) complexed to polyvinyl sulfonate (PVS). If an antibody recognizing a site on PF4:PVS is present, binding will occur. Unbound antibodies are then washed away. An alkaline phosphatase labeled anti-human globulin reagent (Anti-IgG) is added to the wells and incubated. The unbound Anti-IgG is washed away and the substrate PNPP (p-nitrophenyl phosphate) is added. After a 30-minute incubation period, the reaction is stopped with Stopping Solution. The optical density of the color that develops is measured in a spectrophotometer.

REAGENTS

Maximum number of tests per kit:

\begin{itemize}
\item HAT13G: 13 tests per kit
\item HAT45G: 45 tests per kit
\end{itemize}

All reagents should be stored as directed by the label.

| MS | HAT13G: 403574 |
| HTCW | 403624 |
| HSD | 403602 |
| SB | 403613 |
| ESS | 403603 |
| HAG | 403618 |
| PN | 403594 |

Microwell Strips: Flat-bottom microwell strips to which affinity purified platelet factor 4 (PF4) complexed to polyvinyl sulfonate (PVS), has been immobilized. The microwells are enclosed in a resealable foil pouch. Ready for use.

PF4 Concentrated Wash (10X): Tris (hydroxymethyl) aminomethane buffered solution containing sodium chloride and Tween 20. 1% sodium azide. Dilute with deionized or distilled water before use. Store Working Wash solution up to 48 hours at room temperature or up to seven days at 2 to 8°C.

Specimen Diluent: Phosphate buffered saline solution. 0.05% sodium azide. Ready for use.

Substrate Buffer: This solution contains diethanolamine and magnesium chloride. 0.02% sodium azide. Ready for use. Protect from light.

Stopping Solution: Ready for use.

Anti-Human IgG Conjugate: Alkaline phosphatase conjugated goat affinity purified antibody to human immunoglobulin (IgG). 0.1% sodium azide. Dilute in Specimen Diluent before use.

PNPP Substrate: (p-nitrophenyl phosphate) Crystalline powder. Reconstitute with deionized or distilled water and dilute in Substrate Buffer before use. Protect from light.
Positive Serum Control: Human Serum containing bovine albumin. 0.1% sodium azide. Dilute in Specimen Diluent before use.

Negative Serum Control: Human Serum. 0.1% sodium azide. Dilute in Specimen Diluent before use.

**Plate Sealers.**

**PRECAUTIONS**

- Do not use reagents that are turbid or contaminated.
- Care MUST be taken to avoid contamination of Specimen Diluent and Conjugate. Inadvertent contamination of these reagents with human serum will result in the neutralization of the Conjugate and subsequently to test failure.
- Do not use reagents beyond their expiration date.
- Microwells and reagents contained in the kit are not to be used in conjunction with any other test system.
- Substitution of components other than those provided in this kit may lead to inconsistent or erroneous results.
- Discard any unused portions of diluted Conjugate, diluted Positive and Negative Controls, and diluted and reconstituted PNPP reagent after each run.
- When making dilutions, follow pipet manufacturer's instructions for appropriate dispensing and rinsing techniques.
- The enzyme substrate reaction which occurs in the final incubation is temperature sensitive and should be performed in a controlled area at 22 to 25°C.
- Due to variations in instruments or consistently higher or lower room temperatures, it may be necessary for the laboratory to establish a slightly longer or shorter incubation time, in order to consistently achieve valid control results. Because the temperature of the final incubation can affect control values, it is important to periodically monitor the room temperature incubation.

**CAUTION**

- All human serum used in the Positive and Negative controls for this product has been tested and found negative for antibody to HIV, HCV and HBsAg by FDA approved methods. No test method, however, can offer complete assurance that HIV, Hepatitis C virus, Hepatitis B virus or other infectious agents are absent. Therefore, these materials should be handled as potentially infectious.
- Some of the reagents supplied with this kit contain sodium azide as a preservative.
  **WARNING:** Sodium azide reacts with lead and copper plumbing forming highly explosive metal azides. When discarded in a sink, the sink should be flushed with a large volume of water to prevent azide buildup. Sodium azide is a poison and is toxic if ingested.
- Discard all components when completed according to local regulations.

**SPECIMEN COLLECTION AND STORAGE**

Blood should be collected in ACD, or sodium citrate (plasma), or without anticoagulant (serum) using aseptic technique and should be tested while still fresh to minimize the chance of obtaining false positive or false negative reactions due to improper storage or contamination of the specimen. Samples that cannot be tested immediately should be stored at 2 to 8°C for no longer than 48 hours or frozen. Samples frozen at –20°C or below remain in good condition for several years (2-3 years). However, in order to avoid the deleterious effect of repeated freeze/thaw cycles, it is recommended that samples should be aliquoted in small volumes and then stored frozen. Avoid frost-free freezers.

Serum or plasma should be separated from red cells when stored or shipped.

Particulates or aggregates in the sample can cause false positive results or poor duplicate values.

Samples containing particulate matter should be clarified by centrifugation prior to testing.

Only whole human serum or plasma is suitable for this assay. Prior dilution of samples in anything other than normal, ELISA negative human serum could affect the results.

Microbially contaminated, hemolyzed, lipemic, icteric, or heat-inactivated serum samples may give inconsistent test results and should be avoided.

**WARNING:** Samples anticoagulated with heparin should not be used in this assay.

**PROCEDURE**

**Materials Provided**

Vials may contain more reagent than described on the labels. Be sure to measure the reagent with an appropriate device when making dilutions.
1. 4 – 1 x 8 Microwell Strips (HAT13G) or
   12 – 1 x 8 Microwell Strips (HAT45G)
2. 1 x 50 mL PF4 Concentrated Wash (10X)
3. 1 x 30 mL Specimen Diluent
4. 1 x 14 mL Substrate Buffer
5. 1 x 14 mL Stopping Solution
6. 1 x 80 µL Anti-Human IgG Conjugate
7. 4 x 50 mg PNPP Substrate (HAT13G) or
   6 x 50 mg PNPP Substrate (HAT45G)
8. 1 x 100 µL Positive Serum Control
9. 1 x 100 µL Negative Serum Control
10. Plate Sealers

Additional Materials Required
1. Test tubes for patient sample and control dilutions and for reagent dilutions
2. Transfer pipets
3. Adjustable micropipets to deliver 1 – 10 µL, 10 – 100 µL, and 100 – 1,000 µL and disposable tips
4. Timer
5. Microplate reader capable of measuring OD at 405 or 410 and 490 nm
6. Deionized or distilled water
7. Absorbent paper towels
8. Microplate washer or device
9. Centrifuge capable of separating serum or plasma from patient samples
10. 37°C water bath or incubator
11. Heparin, Porcine, USP 10,000 units/mL

Test Procedure
1. Bring all reagents to room temperature.
2. Make Working Wash solution by diluting PF4 Concentrated Wash. Add 1 volume of PF4 Concentrated Wash to 9 volumes of deionized or distilled water. Mix well.
3. Determine the number of patient samples to be tested. Using the Recording Sheet, assign each sample to a location consisting of two (duplicate) wells. Record the identity of each sample on the Recording Sheet.

Prepare Samples and Controls
4. Dilute as follows and mix well:

<table>
<thead>
<tr>
<th>Volume Specimen Diluent</th>
<th>Volume Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC</td>
<td>294 µL</td>
</tr>
<tr>
<td>HNC</td>
<td>294 µL</td>
</tr>
<tr>
<td>Patient Sample</td>
<td>294 µL</td>
</tr>
</tbody>
</table>

**NOTE:** Precise measurement of patient and control samples is essential for accurate results.

5. Remove microwell frame from pouch. Promptly remove and reseal unneeded strips in the protective pouch.

**NOTE:** Only one frame is provided in the kit. Do not discard until all strips have been used.

**NOTE:** Orient the frame with A1 in the top left corner. Be sure that all strips are properly seated and snapped into their frame. Label or number each strip to avoid errors. Maintain the same plate orientation throughout the assay.

6. Add 300 µL of Working Wash solution to all wells and allow to stand at room temperature for 5-10 minutes.

7. Aspirate or decant forcefully and invert on absorbent toweling to prevent drying.

8. Add 50 µL of the appropriate diluted control or sample to the wells as designated on the Recording Sheet.

**NOTE:** Do not add samples or reagents to blank wells.

**NOTE:** If multiple patient samples are tested at the same time, only one set of controls is required. **LABEL EACH STRIP TO AVOID ERRORS.**
9. Seal the microwells with a plate sealer and incubate for 30-35 minutes in a 37°C water bath. If a dry incubator is used instead, increase time by 10 minutes.

10. Dilute the Conjugate 1 to 100 in Specimen Diluent. Use a polypropylene container.

<table>
<thead>
<tr>
<th>Strips</th>
<th>1 or 2 – 1 x 8</th>
<th>4 – 1 x 8</th>
<th>12 – 1 x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAG</td>
<td>10 µL</td>
<td>20 µL</td>
<td>60 µL</td>
</tr>
<tr>
<td>HSD</td>
<td>1.0 mL</td>
<td>2.0 mL</td>
<td>6.0 mL</td>
</tr>
</tbody>
</table>

NOTE: Conjugate is viscous. Prime tip 2-3 times in Conjugate before dispensing and rinse after addition to Specimen Diluent. Mix well.

11. WASH STEP
a) Aspirate or decant contents of each well and blot on absorbent toweling.
b) Add 300 µL Working Wash solution.
c) Aspirate or decant.
d) Repeat steps b + c for a total of 3 or 4 washes.
e) Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

NOTE: It is important to completely remove all wash solution after the final wash.

12. Add 50 µL of diluted Conjugate (made in a previous step) to all wells EXCEPT those designated as BLANKS.

13. Seal the microwells with a plate sealer and incubate for 30-35 minutes in a 37°C water bath. If a dry incubator is used instead, increase time by 10 minutes.

14. Dissolve PNPP Substrate by adding 0.5 mL deionized or distilled water to the vial. Replace stopper and mix well. Protect from light until use.

15. Dilute the PNPP 1 to 100 in the Substrate Buffer.

<table>
<thead>
<tr>
<th>Strips</th>
<th>1 or 2 – 1 x 8</th>
<th>4 – 1 x 8</th>
<th>12 – 1 x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN</td>
<td>20 µL</td>
<td>40 µL</td>
<td>120 µL</td>
</tr>
<tr>
<td>SB</td>
<td>2.0 mL</td>
<td>4.0 mL</td>
<td>12.0 mL</td>
</tr>
</tbody>
</table>

Mix well. Protect from light until use.

16. WASH STEP
a) Aspirate or decant contents of each well and blot on absorbent toweling.
b) Add 300 µL Working Wash solution.
c) Aspirate or decant.
d) Repeat steps b + c for a total of 3 or 4 washes.
e) Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

Proceed promptly through next three steps.

17. Add 100 µL of the diluted PNPP solution to all the wells EXCEPT those designated as BLANKS.

18. Allow the microwells to stand in the dark for 30 minutes at ROOM TEMPERATURE (22 to 25°C).

NOTE: Incubation time and temperature after the addition of PNPP is critical. DO NOT vary the established incubation time or temperature. For consistency, begin timing promptly after addition of the reagent to the first well.

19. Stop the reaction by adding 100 µL of Stopping Solution to each well in the same sequence as the addition of substrate. Add 200 µL of Stopping Solution to the blank wells.

20. Read the absorbance (OD) of each well at 405 or 410 nm using a reference filter of 490 nm. If the results cannot be read immediately, return the wells to a dark location for up to 30 minutes.

21. Subtract the values obtained in the blank wells from all sample and control wells. Many ELISA readers are programmed to automatically perform this step.

22. Record the results on the Recording Sheet.
QUALITY CONTROL

Quality control of PF4 IgG is built into the test system by the inclusion of Positive and Negative Serum Controls. These controls should be included with each test run to help determine if technical errors or reagent failures have occurred.

Criteria for a valid test:

<table>
<thead>
<tr>
<th></th>
<th>Negative Control</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean OD</td>
<td>≤ 0.300</td>
<td>≥ 1.800</td>
</tr>
</tbody>
</table>

OD readings obtained from duplicate tests should fall within 20% of the mean of the two values. Samples whose results are outside of this limit should be re-tested.

NOTE: Poor duplicates can be the result of reagent or sample omission, uneven addition of reagents, uneven temperature during incubations, stray light during the final incubation or cross-well contamination. Failure to test in duplicate may lead to acceptance of erroneous results.

INTERPRETATION OF RESULTS

Test results showing OD values equal to or greater than 0.400 are regarded as positive results.

A close relationship between the OD value of the ELISA result and likelihood that a patient has clinical HIT has been reported by several laboratories.\(^{12,13}\)

The “Confirmatory Procedure”, as described below, should be used to confirm that the antibody reactivity is heparin-dependent. Reports by Whitlatch et al. suggest that performing the high-dose heparin confirmatory procedure might improve the identification of clinically relevant antibodies.\(^{14-16}\)

PROCEDURE FOR CONFIRMATION OF HEPARIN-ASSOCIATED ANTIBODIES

1. To 1 mL of the Specimen Diluent add 10 µL of heparin (10,000 units/mL) to a final concentration of 100 Units per mL.
2. Return to Step 4 above. Dilute patient and positive control samples in the Specimen Diluent containing excess heparin. Also dilute patient, positive, and negative control samples in the Specimen Diluent included in the kit.
3. Re-hydrate strips as before. Add 50 µL aliquots of each patient and control dilution to duplicate wells.

INTERPRETATION OF CONFIRMATORY PROCEDURE

Inhibition of a positive reaction by 50% or more in the presence of excess heparin is considered confirmatory for heparin-dependent antibody characteristic of Type II HIT. The positive control should also show inhibition. The formula for determining % inhibition is as follows:

\[
\text{Inhibition} = \left(1 - \frac{\text{Patient sample with Heparin - Negative Control}}{\text{Patient sample without Heparin - Negative Control}}\right) \times 100 \%
\]

Example: Patient sample gives an OD value of 1.000 in the standard assay with a negative control value of 0.200. With excess heparin, the patient sample gives an OD value of 0.400. Percent inhibition is:

\[
\left(1 - \frac{0.400 - 0.200}{1.000 - 0.200}\right) \times 100 = 75\%
\]

Inhibition of a positive reaction by less than 50% is an equivocal result. This type of reaction is given by a small percentage of antibodies in patients who are suspected of having Type II HIT. The significance of this type of reaction is not yet established. It has not yet been determined whether it is safe to re-administer heparin to patients whose sample gives an equivocal reaction.\(^{17}\)

LIMITATIONS

- Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing or decanting of test wells, exposure of substrate to stray light, omission of test reagents, exposure to higher or lower than prescribed temperature requirements, or omission of steps.
- The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased non-specific binding and produce false-positives in this assay.
- The results of this assay should not be used as the sole basis for a clinical decision.
Some low titer, low avidity antibodies may not be detected using this assay.

The PF4:PVS complexes used in this assay may differ slightly from those created by PF4:heparin. Therefore, it is possible that some antibodies could react with PVS complexes that do not react with heparin complexes and vice versa.

Although a positive reaction obtained using this assay may indicate the presence of a heparin-associated antibody, the detection of such antibodies, however, DOES NOT CONFIRM the diagnosis of heparin-induced thrombocytopenia (HIT).

Some patients may have naturally occurring antibodies to PF4.

Samples from patients exposed to heparin but not on heparin therapy were not used in the evaluation of this product. Therefore, samples from patients other than those on heparin therapy should not be tested.

**PERFORMANCE DATA**

**Precision**

The within run, between run, and total imprecision of the PF4 IgG assay were determined. Three samples of varying antibody concentration were prepared by diluting a serum sample containing a high level of anti-PF4:PVS antibodies into a pool of human serum that contained no anti-PF4:PVS antibodies. The three positive samples and a negative sample were tested in the PF4 IgG assay in duplicate in ten separate assays. To obtain the imprecision of the O.D. values, the data were analyzed by ANOVA according to CLSI Document EP-5A2. The calculations are shown in the table below. The results demonstrated ≤10% CV for the O.D. values for all samples. In addition, the reportable results were analyzed according to CLSI Document EP12-A. There was 100% agreement between the reportable results within run and between run for each sample tested.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean O.D. Value</th>
<th>Within Run SD</th>
<th>Within Run %cv</th>
<th>Between Run SD</th>
<th>Between Run %cv</th>
<th>Total SD</th>
<th>Total %cv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.090</td>
<td>0.005</td>
<td>5.6%</td>
<td>0.008</td>
<td>8.9%</td>
<td>0.009</td>
<td>10.0%</td>
</tr>
<tr>
<td>Low Positive</td>
<td>0.562</td>
<td>0.023</td>
<td>4.1%</td>
<td>0.056</td>
<td>10.0%</td>
<td>0.058</td>
<td>10.3%</td>
</tr>
<tr>
<td>Medium Positive</td>
<td>1.613</td>
<td>0.035</td>
<td>2.2%</td>
<td>0.075</td>
<td>4.7%</td>
<td>0.079</td>
<td>4.9%</td>
</tr>
<tr>
<td>High Positive</td>
<td>2.701</td>
<td>0.069</td>
<td>2.6%</td>
<td>0.092</td>
<td>3.4%</td>
<td>0.092</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

**PF4 IgG Normal Range**

One hundred twenty serum samples were obtained from normal healthy individuals and were tested (in duplicate) in the PF4 IgG. The O.D. values did not show a normal distribution and therefore a non-parametric analysis was used to determine the normal range distribution of O.D. values (95% reference interval with a 90% confidence). The upper end of the normal range was calculated to be 0.352 O.D. units.

**Method Comparison: Comparison of PF4 IgG to PF4 Enhanced assay and Serotonin Release Assay (SRA).**

Two independent studies were conducted in which PF4 IgG was compared to both the PF4 Enhanced assay and the Serotonin Release Assay (SRA). The PF4 Enhanced assay is a qualitative ELISA for the detection of IgG, IgA, and IgM heparin associated antibodies. A total of 400 serum samples were tested (Site 1; n = 229 samples, Site 2; n = 171 samples). The following tables show the analysis of the method comparisons for the combined data from the two studies.

**PF4 Enhanced assay**

<table>
<thead>
<tr>
<th>PF4 IgG</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>77</td>
<td>0</td>
<td>77</td>
</tr>
<tr>
<td>Negative</td>
<td>52</td>
<td>271</td>
<td>323</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>271</td>
<td>400</td>
</tr>
</tbody>
</table>

Agreement: 87%
Co-positivity: 60% (95% Confidence Interval = 51.1 – 67.8%)
Co-negativity: 100% (95% Confidence Interval = 98.6 – 100.0%)

**Serotonin Release Assay**

<table>
<thead>
<tr>
<th>PF4 IgG</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>41</td>
<td>36</td>
<td>77</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>319</td>
<td>323</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>355</td>
<td>400</td>
</tr>
</tbody>
</table>

Agreement: 90%
Co-positivity: 91% (95% Confidence Interval = 79.3 – 96.5%)
Co-negativity: 90% (95% Confidence Interval = 86.3 – 92.6%)
Of the four samples which were negative in the PF4 IgG assay and positive in the SRA, three of these samples were found to contain IgM or IgA antibodies reactive with the PF4:PVS complex. The remaining sample was found to be negative in the SRA when tested by the SRA in another laboratory.

**Interfering Substances**

The following endogenous substances were tested in the PF4 IgG assay at the concentration indicated. Testing was performed according to CLSI EP7: Interference Testing in Clinical Chemistry: Approved Guideline. Each substance was spiked into samples containing varying reactivity of PF4:heparin antibodies (negative, low – , medium –, and high – positive). The samples were then tested in the PF4 IgG assay. The results were compared to those of the control in which no interfering substance was added. For all samples tested, the substances had no significant effect (< 10% difference in O.D. values between the test sample and control) on the results obtained in the PF4 IgG assay.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
</tbody>
</table>

**Cross-Reactive Substances**

In order to determine possible cross-reactivity between the target antigen and antibodies other than heparin-associated antibodies, 68 samples containing a variety of antibodies which included known antibodies to platelet alloantigens, platelet autoantibodies, antibodies to HLA class I and anti-rheumatoid factor were tested in this assay and none were found to cross react with the target antigen immobilized in the microwells.

**REFERENCES**

<table>
<thead>
<tr>
<th>Warning</th>
<th>Danger</th>
</tr>
</thead>
<tbody>
<tr>
<td>H302</td>
<td>Harmful if swallowed</td>
</tr>
<tr>
<td>H318</td>
<td>Causes serious eye damage.</td>
</tr>
<tr>
<td>H412</td>
<td>Harmful to aquatic life with long lasting effects</td>
</tr>
<tr>
<td>EUH032</td>
<td>Contact with acids liberates very toxic gas</td>
</tr>
<tr>
<td>P264</td>
<td>Wash hands thoroughly after handling</td>
</tr>
<tr>
<td>P270</td>
<td>Do not eat, drink or smoke when using this product</td>
</tr>
<tr>
<td>P273</td>
<td>Avoid release to the environment</td>
</tr>
<tr>
<td>P280</td>
<td>Wear protective gloves/protective clothing / eye protection / face protection</td>
</tr>
<tr>
<td>P301 + P312</td>
<td>IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.</td>
</tr>
<tr>
<td>P305 + P351 + P338</td>
<td>IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</td>
</tr>
<tr>
<td>P310</td>
<td>Immediately call a POISON CENTER or doctor/physician.</td>
</tr>
<tr>
<td>P330</td>
<td>Rinse mouth</td>
</tr>
</tbody>
</table>