**INTRODUCTION**

One of the most prevalent disorders of man is the dietary deficiency of iron resulting in anaemia. Therefore, the assays of iron total binding capacity and other assessments of iron compounds in the body are clinically significant.

Iron storage compounds in the body include haemoglobin, haemosiderin, myoglobin and the cytochromes. Ferritin, a major iron storage protein, is found in the liver, spleen and bone marrow. Methods previously used to measure iron in such tissues were invasive, caused patient trauma and lacked adequate sensitivity.

**PATHOZYME FERRITIN** is an Enzyme Immunoassay (EIA) for the quantitative determination of Ferritin in human serum.

For professional use only.

**PRINCIPLE OF THE TEST**

Specific anti-Ferritin antibodies are coated on to microtiter wells. Test sera are applied. Then monoclonal anti-Ferritin labelled with Horseradish Peroxidase enzyme (Conjugate) is added. If human Ferritin is present in the sample, it will combine with the antibody on the well and the enzyme Conjugate, resulting in the Ferritin molecules being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed with distilled water to remove unbound labelled antibodies. Addition of the Substrate (TMB), a colour will develop only in wells are washed with distilled water to remove unbound labelled antibodies. After incubation, the wells are washed with distilled water to remove unbound labelled antibodies. Addition of the Substrate (TMB), a colour will develop only in wells in which antigen (Ferritin) is present.

**INTENDED USE**

**PATHOZYME FERRITIN** is an Enzyme Immunoassay (EIA) for the quantitative determination of Ferritin in human serum. For professional use only.

**CONTENTS**

- Microtiter Plate
- TMB (Colours)
- BSA Buffer (Colours)
- Anti Ferritin HRP Conjugate
- Stop Solution
- Reference Standard: Ferritin diluted in purified water
- Disposable pipette tips (100, 200, 500 and 1000μl)
- Absorbent paper
- Microplate reader fitted with a 490nm filter
- Graph paper

**MATERIAL REQUIRED BUT NOT PROVIDED**

- Microplate wells coated with specific antibody contained in a resealable foil bag with a desiccant
- 12 x 2 wells/tube

**SPECIMEN COLLECTION AND PREPARATION**

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminted or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing.

If longer storage is required, store at -20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Serum samples, with no additives, are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at -20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not repeatly freeze-thaw the specimens as this will cause false results.

**REAGENT PREPARATION**

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

**ASSAY PROCEDURE**

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
2. Prepare 100μl of Anti-Ferritin HRP Conjugate to 500μg/ml in distilled water. Ready to use. (Colours)
3. Prepare 100μl of Stop solution in purified water. Ready to use (Colours)

**PROCEDURE**

1. Dispense 50μl of Anti-Ferritin HRP Conjugate into each well.
2. Thoroughly mix for 30 seconds. It is very important to have a complete mixing at this stage.
3. Incubate the plate for 45 minutes at room temperature (20°C to 25°C).
4. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
5. Wash 5 times. Place the wells sharply against absorbent paper. Wash the empty wells 5 times.
6. Strike the wells sharply against absorbent paper or paper towel to remove all residual droplets.

**PRECAUTIONS**

- Do not freeze any of the reagents (except Standards for storage) as this will cause irreversible damage.
- Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

**STORAGE**

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label.

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and component labels. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

Do NOT FREEZE ANY OF THE REAGENTS (except Standards for storage) as this will cause irreversible damage.

- All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

**LIMITATIONS OF USE**

The use of samples other than serum has not been validated in this test.

There is no reuse protocol for this product.

When making an interpretation of the test it is strongly advised to take all clinical data into consideration, diagnosis should not be made solely on the findings of one clinical assay.
11. Machine Washing: Ensure that 300μl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by sucking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

12. Dispense 100μl Substrate Solution into each well and mix gently for 5 seconds.

13. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).

14. Stop the reaction by adding 100μl Stop Solution to each well.

15. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow

16. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Specimens and standards should be run at the same time to keep testing conditions the same.

These kits were shown to give good correlation.

Abbott AxSYM Mean

Number of Samples 98

Intercept 1.013

Slope 0.999

Correlation coefficient 0.993

Intercept 1.013

Slope 0.999

Correlation coefficient 0.999

The co-efficient of variation of PATHOZYME FERRITIN is less than or equal to 10%.

In an evaluation between the Omega Pathzyme Ferritin kit and the Abbott AxSYM Ferritin assay K0 for samples with levels between 1.0 and 831 ng/ml the following data was generated.

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<th>Number of Samples</th>
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<tr>
<td>Intercept</td>
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</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
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</tbody>
</table>

These kits were shown to give good correlation.

REFERENCES


QUICK REFERENCE TEST PROCEDURE

1. Dispense 20μl of Test Serum or Standards and 100μl of Anti-Ferritin HRP Conjugate into each well and mix thoroughly for 30 seconds.

2. Incubate for 45 minutes at room temperature (20°C to 25°C).

3. Discard well contents and wash 5 times with distilled water.

4. Add 100μl of Substrate Solution to each well. Gently shake for 5 seconds.

5. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).

6. Add 100μl of Stop Solution to each well and gently shake for 30 seconds.

7. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

8090 ISSUE 6 Revised June 2017 © Omega Diagnostics Ltd. 2017