## WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose. In the event of performance changes or product malfunction, please contact manufacturer.

## BIBLIOGRAPHY


1. Stabler SP. Vitamin B12 deficiency [J]. N Engl J Med, 2013, 3(1):54-55.
2. Arendt J, Nexo E. Unexpected high plasma cobalamin/Proposal for a diagnostic strategy[J]. Clinical Chemistry & Laboratory Medicine, 2013, 51(3):489-496.
3. Liu B, Liu Z, Jing G. Fluorescence Resonance Energy Transfer Between Acridine Orange and Rhodamine B and Analytical Application on Determination of Vitamin B12[J]. Analytical Letters, 2005, 38(9):1367-1377.


## SYMBOL KEYS

| | | | |
|---|---|---|---|
| Temperature Limitation | Consult Instructions for use | Date of Manufacture | Do not reuse |
| Manufacturer | IVD *In vitro* Diagnostic Medical Device | This side up | Use by |
| Contains sufficient for <n> tests | REF Catalogue Number | LOT Batch Number / Lot Number | DEVICE Device |


Manufactured by:

**Zephyr Biomedicals**

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

**Regd. Office:** Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

Website: www.tulipgroup.com Email: sales@tulipgroup.com

0323/VER-01


# FIAcheck™

# Vitamin-B12

**Fluorescence Immunoassay for Quantitative determination of Vitamin-B12 in Human Serum, Plasma and Whole blood**

**DEVICE**

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 4°C to 30°C

## INTENDED USE

**FIAcheck™ Vitamin-B12** Fluorescence Immunoassay is intended for the in-vitro quantitative measurement of Vitamin-B12 in human Serum, Plasma and whole blood.

## INTRODUCTION

Suitable for the in vitro quantitative determination of vitamin B12 in human serum, plasma and whole blood. Vitamin B12 (VB12), also known as cobalamin, is a natural vitamin with a molecular weight of 1355.37Da and is an important biological cofactor and nutrient that cannot be synthesized in the human body but could be obtained from animal diets. Deficiency of VB12 is mainly reflected in the blood and nervous system and can lead to diseases such as pernicious anemia, dementia and mental depression. VB12 is an essential component of DNA synthesis and its deficiency can lead to abnormal DNA synthesis and megaloblastic anemia, as well as being essential for the formation and maintenance of myelin sheaths, and its deficiency can cause nerve injury, leading to various neurological disorders. In addition, the elevation of VB12 levels are a marker for certain diseases, including renal failure, liver disease, diabetes and haematological diseases. Common detection methods are used clinical laboratory tests which include chemiluminescence and immunofluorescence assays.

## PRINCIPLE OF THE TEST

The **FIAcheck™ Vitamin-B12** is based on principle of agglutination of antibodies/anti-sera with respective antigen in immune-chromatographic format using fluorophores as signal generators. The **FIAcheck™ Vitamin-B12** test device is coated with immobilized VB12 antigen on the test line, sheep anti chicken IgY in control line and a mixture of VB12 sheep monoclonal antibody and chicken IgY labeled with fluorescent microspheres on binding pad.

VB12 is released from the sample by aspirating the sample into the diluent and the treated sample is added on the reagent device.

VB12 in sample binds to the VB12 sheep monoclonal antibody labeled with fluorescent microspheres in the binding pad. The fluorescent labeled Ag-Ab complex moves forward due to capillary action and is captured by the immobilized VB12 antigen forming a double antibody sandwich and produces the test line. Chicken IgY labelled with fluorescent microspheres binds with sheep anti chicken IgY to produce the control line. When the **FIAcheck™** test device is inserted in the **FIAcheck™** analyzer, it scans both the test line and control line. The ratio of the two fluorescence values is used to calculate the concentration of the analyte present in the sample.

## MATERIALS AND COMPONENTS

***Materials provided with the test kits:***

- **FIAcheck™ Vitamin-B12** test device in a sealed pouch with desiccant.
- QR Code card for calibration.
- Sample Diluent. Ready to use.
- Empty vials for sample dilution.

***Materials required but not provided***

- Precision pipettes: 100-1000µl
- Disposable pipette tips
- Disposable Gloves
- **FIAcheck™** Analyzer (Time Resolved Fluorescence Immunoassay Analyzer)
- Digital Thermometer
- Stopwatch

## STORAGE AND STABILITY

1. **FIAcheck™ Vitamin-B12** kit is stable at 4°C to 30°C upto expiry date printed on the label. DO NOT FREEZE.
2. **FIAcheck™** Test device should be used within 30 minutes once the foil pouch is opened.
3. If the colour of the desiccant has changed from blue to pink or colourless at the time of opening the pouch, kindly discard the device and use another device.

4. Once opened, the sample diluent can be stored between 4°C to 30°C for remaining duration of its shelf-life.

## SAMPLE COLLECTION

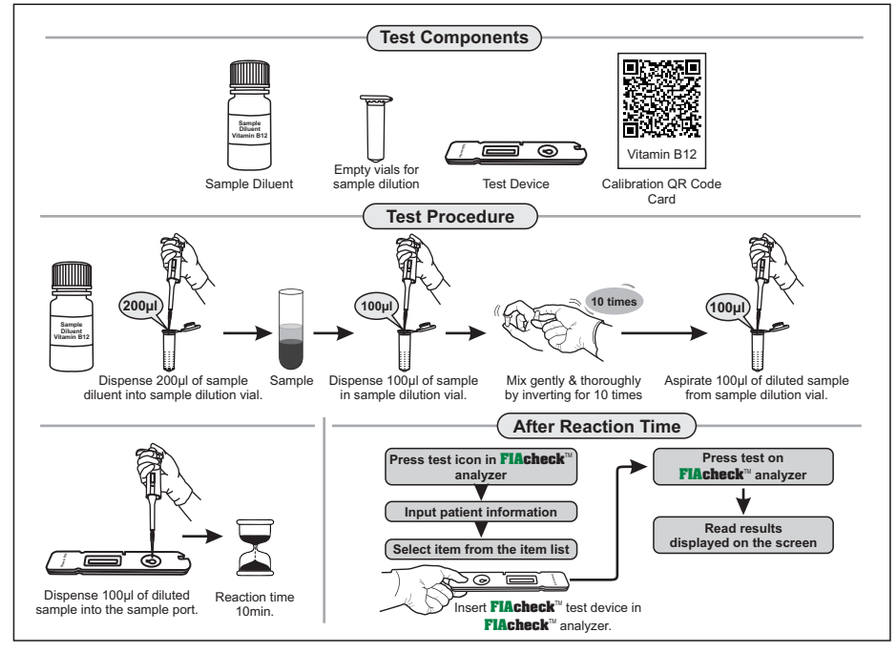
1. Only Human Serum, Plasma or Whole blood should be used. Other bodily fluids and samples may not give accurate result.
2. Plasma can be anti-coagulated with Heparin and Sodium citrate or Tri sodium citrate under aseptic conditions.
3. Whole blood can be anti-coagulated with EDTA under aseptic conditions.
4. The test should be performed within 4 hours after the sample collection at room temperature.
5. Avoid grossly hemolytic, lipemic or turbid serum/plasma samples. Do not use clotted or hemolysed whole blood samples.
6. Preferably use fresh samples. However, samples can be stored for 3 days at 2°C to 8°C, and if more than 3 days, they should be stored at -20°C. Whole blood samples should be used immediately and should not be frozen.
7. The sample should be recovered to room temperature (18°C to 30°C) before testing. Avoid repeated freezing and thawing of samples as it can affect the test values.
8. Samples containing precipitate or particulate matter should be clarified by centrifugation prior to use.
9. Samples should be free from particulate matter and microbial contamination.

## PRECAUTIONS

1. Only for In vitro diagnostic use.
2. Bring all reagents and samples to room temperature before use.
3. After the test device is removed from the sealed pouch, it should be used immediately or within 30 minutes of opening the pouch.
4. Do not reuse the tested **FIAcheck**™ device. Do not use sample dilution vial for more than one sample.
5. Do not use damaged **FIAcheck**™ test device or pouch.
6. All samples should be considered potentially infectious and discarded appropriately as per Standard Bio-Safety guidelines.
7. Do not use kit after the expiry date.
8. Do not mix components of one kit with another.
9. Always use new tip for each sample and reagent.
10. Scan QR code card specific to the lot you are using.
11. Ambient temperature of testing environment directly impacts the accuracy of results. Ideal working temperature is 18°C to 30°C.
12. It is highly recommended to mix the sample diluent and sample mixture thoroughly by gently inverting the vial 10 times. (Refer pictorial presentation.)
13. It is not recommended to use the sample diluent and sample mixture beyond specified time.
14. The **FIAcheck**™ test device should be read immediately after the specified reaction time. Delay in reading might affect the accuracy of results.
15. The **FIAcheck**™ test device should be used only in conjunction with **FIAcheck**™ analyzer for accurate and reliable results.

## TEST PROCEDURE

1. To calibrate the **FIAcheck**™ **Vitamin-B12** kit, scan the QR code card provided with the kit.
2. Dispense **200** µl of sample diluent into the empty sample dilution vial.
3. Add **100**µl of the test sample into this sample diluent & mix by rinsing the tip 3 times.
4. Close the lid of the sample dilution vial, label with sample identity and mix the content of the vial by gently inverting it for 10 times. (See pictorial representation).
5. Remove **FIAcheck**™ **Vitamin-B12** test device from sealed pouch and place it horizontally on a clean table, label the device with sample identity.
6. Dispense 100 µl of the above mixture at the sample port in the **FIAcheck**™ **Vitamin-B12** test device.
7. Incubate at room temperature (18°C to 30°C) for **10 minutes**.
8. After 10 minutes, insert the test device immediately into the **FIAcheck**™ analyzer and read results.




**Expected Range**

The following reference intervals were derived from a 95% confidence interval statistical analysis of serum and plasma VB 12 levels in 196 healthy individuals:

Vitamin B12: 197-771 pg/mL

Note: Due to geographical, ethnic, gender and age differences, it is recommended that each laboratory establish its own reference range.

## PERFORMANCE CHARACTERISTICS

1. Measuring Range: 50pg/mL-2000pg/mL
2. Lower Detection Limit: ≤50pg/mL
3. Upper Detection Limit: ≥2000pg/mL
4. Accuracy: Based on comparison experiments, the relative standard deviation of ±15%, and the correlation coefficient of r≥0.990 was observed.
7. Hook Test: No hook effect with high concentration sample. Hook test was conducted with reference material exceeding the upper limit of measuring range, and the detection result was greater than the upper limit of detection.
8. In an internal study, **FIAcheck**™ **Vitamin-B12** was evaluated against commercially available licensed kit with 100 random clinical samples and **FIAcheck**™ **Vitamin-B12** has demonstrated 100% clinical correlation with the commercially available licensed kit.

| Vitamin-B12 Levels | No. of samples | FIAcheck™ Vitamin-B12 | EIA Vitamin-B12 |
|---|---|---|---|
| Normal | 80 | 80 | 80 |
| Low | 8 | 8 | 8 |
| High | 12 | 12 | 12 |

9. In an external Study, **FIAcheck**™ **Vitamin-B12** has been evaluated by a NABL accredited lab against their reference method. In this evaluation **FIAcheck**™ **Vitamin-B12** has demonstrated 100% correlation with the reference method.
   *Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics (P) Ltd).

## LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. This kit is only for human whole blood, serum and plasma.