

# FACTOR VIII ANTIBODY SCREEN

New!

## ELISA for the detection of IgG antibodies to Human Factor VIII

### GENERAL INFORMATION

Factor VIII (FVIII) is an important mediator within the coagulation cascade. A deficiency in FVIII, either in its quantity or function, causes Hemophilia A. Hemophilia A occurs in 1 out of every 5000 live male births. Depending on the severity of the FVIII deficiency, Hemophilia A can be manifested as a mild, moderate, or severe bleeding disorder with symptoms ranging in severity from excessive bleeding following minor surgery or trauma to life-threatening spontaneous hemorrhages and soft tissue bleeds. In rare cases, a form of acquired Hemophilia A can be caused by spontaneous generation of autoantibodies against endogenous FVIII.

The development of antibodies against human Factor VIII (FVIII) is one of the most detrimental complications in the treatment of hemophilia A, as well as in patients with acquired hemophilia. The predominately IgG anti-FVIII antibodies can bind to the FVIII in such a way as to block the interactions necessary for FVIII procoagulation activity. These inhibitory antibodies develop in approximately 25% of patients with moderate and severe hemophilia A and can lead to the direct neutralization of any FVIII administered as therapy.<sup>1</sup> In addition, antibodies are also developed to epitopes not associated with FVIII activity. These non-inhibitory antibodies may increase the clearance of FVIII from the circulation, reduce binding to its carrier protein (VWF) or even directly hydrolyze the FVIII molecule.<sup>2,3,4</sup>

According to the Association of Hemophilia Centre Directors of Canada, all Hemophilia A patients who have received Factor VIII infusions as well as those patients who are suspected of having acquired Hemophilia A should be screened for the presence of anti-FVIII antibodies.<sup>5</sup>

### PRINCIPLE OF THE PROCEDURE

The Factor FVIII Antibody Screen is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies reactive with recombinant human factor VIII (FVIII) in human serum and plasma.

Patient sample is added to microwells coated with recombinant FVIII molecules allowing antibody, if present, to bind. Unbound antibodies are (over)

### FEATURES & BENEFITS

- Qualitative screening assay for antibodies directed against human Factor VIII
- Solid-phase ELISA using immobilized full-length recombinant human FVIII as a target
- Designed to detect inhibitory and non-inhibitory IgG antibodies
- Faster than the Bethesda assay -- up to 44 samples in duplicate can be screened in 2 hours
- Utilizes half-area microwells to minimize patient sample usage -- requires only 8 $\mu$ L of sample
- Can use either serum or plasma as the sample source
- **Coming Soon!** E-QUATE™ Quantitative FVIII Antibody Assay

### KIT COMPONENTS

- Microwell strips coated with recombinant full-length human Factor VIII
- Wash Buffer
- Specimen Diluent
- Positive, Negative and Kit Controls
- Conjugate
- Colorimetric Detection System

### ORDER INFORMATION

|                 |                          |
|-----------------|--------------------------|
| CATALOG NO:     | F8S                      |
| DESCRIPTION:    | FVIII Antibody Screen    |
| SIZE:           | Maximum 44 Tests Per Kit |
| AVG SHELF LIFE: | 2 Years                  |
| STORAGE:        | 2-8°C                    |

For In Vitro Diagnostic Use.



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**PRINCIPLE OF THE PROCEDURE (CONTINUED)**

then washed away. An alkaline phosphatase labeled anti-human immunoglobulin 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 by a sodium hydroxide solution. The optical density of the color that develops is measured in a spectrophotometer.

**COMPARISON OF FACTOR VIII ANTIBODY SCREEN TO BETHESDA ASSAY**

Two hundred and six samples were tested on the Factor VIII Antibody Screen and in the Bethesda Assay or a modified Bethesda Assay (Bethesda Screen). The Bethesda Assay is considered the gold standard for the quantitative measurement of inhibitory antibodies to Factor VIII. Any sample with a positive Bethesda titer was assigned a positive reportable result and any sample with a negative Bethesda titer was assigned a negative reportable result. The following table shows the results from this study:

|                                    |          | Bethesda Assay |          |       |
|------------------------------------|----------|----------------|----------|-------|
|                                    |          | Positive       | Negative | Total |
| <b>Factor VIII Antibody Screen</b> | Positive | 92             | 12       | 104   |
|                                    | Negative | 4              | 98       | 102   |
|                                    | Total    | 96             | 110      | 206   |

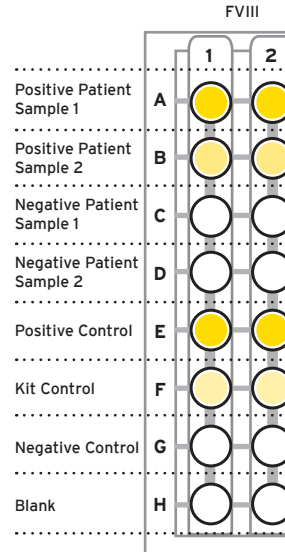
|                |  |
|----------------|--|
| Agreement:     | 92.2%  |
| Co-positivity: | 95.8% (95% Confidence Interval = 89.8 - 98.4%) |
| Co-negativity: | 89.1% (95% Confidence Interval = 81.9 - 93.6%) |

The 4 samples that were Bethesda positive and Factor VIII Antibody Screen negative ranged in Bethesda titer from 0.8 to 3 BU. The Bethesda values were not confirmed in a second laboratory, however the Factor VIII Antibody Screen results were consistently negative for these samples. Twelve samples were shown to be Bethesda negative and Factor VIII Antibody Screen positive. The majority of these samples were from serial tests of the same patient, monitoring either the development of Factor VIII antibodies or the reduction in Factor VIII antibodies during immune tolerance therapy. In general, these discrepancies were found in samples taken from the patient immediately prior to a patient becoming Bethesda positive or immediately following a patient becoming Bethesda negative, suggesting an increased sensitivity to Factor VIII antibodies in the GTI Factor VIII Antibody Screen. The Factor VIII Antibody Screen showed excellent sensitivity (co-positivity), specificity (co-negativity), and overall agreement when compared to the gold standard, Bethesda Assay.

**Interfering Substances**

The following substances showed no interference in the Factor VIII Antibody Screen at the concentrations indicated:

|                     |            |
|---------------------|------------|
| Hemoglobin          | < 500mg/dL |
| Bilirubin           | < 20mg/dL  |
| Intralipid          | < 500mg/dL |
| Gammagard (IVIG)    | < 200µg/dL |
| Rituxan (rituximab) | < 10µg/dL  |

**RESULTS****REFERENCES**

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20925 Crossroads Circle, Suite 200  
Waukesha, WI 53186 USA

IN USA 800. 233 . 1843  
TEL 262 . 754 . 1000  
FAX 262 . 754 . 9831  
EMAIL [gti@gtidiagnostics.com](mailto:gti@gtidiagnostics.com)  
WWW [gtidiagnostics.com](http://gtidiagnostics.com)

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