**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**

**A. 510(k) Number:**
   k061794
**B. Purpose for Submission:**
   New Device
**C. Measurand:**
   Thyroglobulin (TG) and Thyroperoxidase (TPO) autoantibodies
**D. Type of Test:**
   Multiplex bead-based flow cytometric immunoassay
**E. Applicant:**
   Biomedical Diagnostics S.A. (bmd)
**F. Proprietary and Established Names:**
   FIDIS™ THYRO
**G. Regulatory Information:**
   1. Regulation section:
      21 CFR 866.5660 Multiple autoantibodies immunological test system
      21 CFR 866.5870 Thyroid autoantibody immunological test system
   2. Classification:
      Class II
   3. Product code:
      JZO, System, Test, Thyroid Autoantibodies
      JNL, Immunochemical, Thyroglobulin Autoantibodies
   4. Panel:
      Immunology (82)
**H. Intended Use:**
   1. Intended use(s):
      The FIDIS™ THYRO kit is a semi-quantitative homogeneous fluorescent-based
      microparticles immunoassay using flow cytometry readings.  It is designed for the
      detection of antibodies directed against thyroperoxidase (TPO) and thyroglobulin
      (TG).  The FIDIS™ THYRO kit uses serum only and is to be run on the FIDIS
      Analyzer, MLX-BOOSTER™ Software and Washer.
   2. Indication(s) for use:
      The test system is used as an aid in the diagnosis of autoimmune thyroid
      pathologies (Graves' disease and Hashimoto's thyroiditis), in conjunction with
      clinical findings and other laboratory tests.
   3. Special conditions for use statement(s):
      Prescription Use only.
   4. Special instrument requirements:
      The FIDIS™ THYRO is to be used as part of the FIDIS™ Instrument System
      (Luminex 100™ plus FIDIS™ MLX-Booster Software)
      Caris™System (diluting/dispensing device), optional
**I. Device Description:**
   The device consists of the following:  color-coded microspheres covalently coupled
   to either thyroglobulin or thyroperoxidase (ready-to-use); goat anti-human IgG

coupled to phycoerythrin (ready-to-use), a calibrator titered for each specificity (ready-to-use); a positive control IgG (to be diluted), a negative control (to be diluted), and 10x concentrated PBS-Tween.

**J. Substantial Equivalence Information:**
1. Predicate device name(s):
   General Biometrics, Inc ImmunoWELL™ Thyroglubulin Test
   General Biometrics, Inc ImmunoWELL™ TPO (Microsome) Test
2. Predicate 510(k) number(s):
   k905485 (TG); k905486 (TPO)
3. Comparison with predicate:

| Similarities | | | |
|---|---|---|---|
| Feature | New Device | Predicate | Predicate |
| | FIDIS™ THYRO | ImmunoWELL™ TPO Antibodies | ImmunoWELL™ TG Antibodies |
| Intended Use | Determination of antibodies against TPO and TG | Determination of antibodies against TPO | Determination of antibodies against TG |
| Antigen | TPO: recombinant human thyroid peroxidase. TG: natural protein isolated from human thyroid glands | Recombinant human thyroid peroxidase | Purified human thyroglobulin |
| Sample type | Serum | Same | Same |
| Type of test | Semiquantitative | Same | Same |
| Controls | Positive and Negative using diluted human serum | Same | Same |

| Differences | | | |
|---|---|---|---|
| Feature | New Device | Predicate | Predicate |
| Assay Type | Flow Cytometry | ELISA | ELISA |
| Solid Phase | Color-coded microspheres | Microtiter plate | Microtiter plate |
| Assay Format | Multiplex | Individual analytes | Individual analytes |
| Sample Dilution | 1:200 | 1:100 | 1:100 |
| Reporter Conjugate | Phycoerythrin | HR Peroxidase | HR Peroxidase |
| Substrate Solution | None | TMB | TMB |
| Detection Method | Fluorescence/Flow cytometer | Colorimetry/ Spectrophotometer | Colorimetry/ Spectrophotometer |
| Diagnostic Values: Negative: Equivocal: Positive: | <130 IU/mL 130 -150 IU/mL >150 IU/mL | <45 IU/mL 45-65 IU/mL >65 IU/mL | <85 IU/mL 85-120 IU/mL >120 IU/mL |

| Differences | | | |
|---|---|---|---|
| Feature | New Device | Predicate | Predicate |
| Calibrator | 1 concentration used to interpolate each antigenic specificity | 5 ready-to-use dilution | 5 ready-to use dilutions |
| Traceability | Anti-thyroid microsome serum WHO 66/387 and anti-thyroglubulin serum WHO 65/93 | Unknown | Unknown |

**K. Standard/Guidance Document Referenced (if applicable):**
Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices

**L. Test Principle:**
The FIDIS™ THYRO kit is a multiplexed, semiquantitative, fluorescent immunoassay performed on the FIDIS™ System using MLX-BOOSTER Software, and allows the detection and identification of antibodies against thyroglubulin (TG) and thyroperoxidase (TPO). Diluted patient sera and microsphere suspensions precoated with either TPO or TG are mixed in a 96 well microtiter plate. TPO or TG specific antibodies in the patient sera, if present, bind to the immobilized antigen on the beads. Any unbound material is removed by performing a filtration wash step. Phycoerythrin-conjugated goat anti-human IgG is added to the plate and a further incubation performed. The conjugated anti-human IgG binds to the TPO or TG specific antibodies immobilized on the microsphere surface to form an antigen/antibody complex. The bead suspension is then analyzed by the FIDIS instrument. The FIDIS™ Instrument uses a red diode laser beam to distinguish between the internally, color-coded microspheres for each antigen on the basis of its unique fluorescence intensity (red to orange), while a green laser beam excites the reporter conjugate, quantifying the fluorescence of the antibody captured by each microsphere. The degree of binding is calculated in biological units (IU/mL) using specific data software (MLX-BOOSTER). Measurement of the fluorescent signal from the final reaction allows the quantification of the presence or absence of autoantibodies.

**M. Performance Characteristics (if/when applicable):**
1. Analytical performance:
   a. *Precision/Reproducibility:*
      To evaluate intra-assay and inter-assay precision and reproducibility, a total of 14 serum samples with various concentrations of antibodies to TPO or TG representing the measuring range, were analyzed using the FIDIS™ THYRO kit. For within-run, 6 TPO samples and 8 TG samples representing the measuring range were assayed 10 times in one run. For between-run, the same samples were assayed 2 times per run for 6 runs. The acceptance criteria %CV <30% was achieved. The results are as follows:

| Antigen | Within-run (10 tests in the same run) | | Between-run (2 tests in 6 different runs) | |
|---|---|---|---|---|
| | Mean value | CV (%) | Mean value | CV (%) |
| **TPO** | 135 | 9 | 131 | 8 |
| | 194 | 3 | 169 | 12 |
| | 215 | 7 | 195 | 12 |
| | 351 | 6 | 309 | 13 |
| | 612 | 3 | 562 | 7 |
| | 935 | 6 | 890 | 9 |
| **TG** | 109 | 4 | 114 | 11 |
| | 110 | 10 | 111 | 10 |
| | 125 | 4 | 125 | 9 |
| | 180 | 7 | 169 | 11 |
| | 194 | 7 | 182 | 10 |
| | 396 | 5 | 334 | 15 |
| | 730 | 3 | 645 | 10 |
| | 1395 | 5 | 1292 | 9 |

Precision and reproducibility of the assays using the optional automated CARIS™ system (diluting and dispensing device) was determined.  A total of 11 serum samples (5 TPO and 5 TG) were assayed for within-run (10 times in one run) and between-run (4 times in 6 different runs). Results are as follows:

| Antigen | Within-run (10 tests in the same run) | | Between-run (4 tests in 6 different runs) | |
|---|---|---|---|---|
| | Mean value | CV (%) | Mean value | CV (%) |
| **TPO** | 142 | 8 | 142 | 7 |
| | 188 | 6 | 178 | 8 |
| | 379 | 4 | 375 | 13 |
| | 540 | 6 | 501 | 12 |
| | 1013 | 6 | 1020 | 5 |
| **TG** | 121 | 9 | 114 | 13 |
| | 144 | 12 | 144 | 12 |
| | 268 | 4 | 261 | 8 |
| | 314 | 8 | 302 | 17 |
| | 549 | 8 | 543 | 9 |
| | 1838 | 5 | 1763 | 7 |

b.  *Linearity/assay reportable range:*
   Linearity is not claimed for this assay.
c.  *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   Calibrator titers are expressed in International Units per mL (IU/mL) and are standardized against International Reference 66.387 WHO for anti-thyroperoxidase autoantibodies and International Reference 65/93 WHO for anti-thyroglobulin autoantibodies.
d.  *Detection limit:*

Not Applicable
e. *Analytical specificity:*
Cross-reactivity with autoimmune antibodies common to other systemic autoimmune diseases was evaluated by testing 96 serum samples representing a spectrum of autoimmune diseases.  Eight of the 96 samples were positive for TPO and 2 were positive for TG.  The Limitations section of the package insert includes the statement "Hemolytic, lipemic, icteric or citrated samples, or samples with abnormal concentration of immunoglobulins, cryoglobulinemia or complement levels or samples with rheumatoid factor may confound the results of this assay.  Use of these samples should be avoided."

The autoimmune disorders evaluated and results are as follows:

| Crossreactive samples | Number of tested sera | Number of positive sample | |
|---|---|---|---|
| | | TPO | TG |
| Cryoglobulin | 8 | | |
| Complement | 30 | 1 | 1 |
| Hypergammaglobulin | 13 | 4 | |
| IgG monoclonal immunoglobulins | 5 | | |
| IgM monoclonal immunoglobulins | 17 | 1 | |
| Rheumatoid factor | 9 | 1 | 1 |
| Hemolyzed sera | 6 | | |
| Citrated plasma | 6 | 1 | |
| CIC | 1 | | |
| Lipemic plasma | 1 | | |

f. *Assay cut-off:*
The assay cut-off of 150 IU/mL was determined by assaying 146 normal serum samples (50 normal and 96 with potential biological interferences).  The following results were obtained and between the thresholds the results are considered equivocal (results $\leq$ 150 IU/mL and $\geq$ 130 IU/mL).

| Percentiles of the distribution values | <130 IU/mL | <150 IU/mL |
|---|---|---|
| TPO | 91.8% (134/146) | 92.5% (135/146) |
| TG | 98.6% (144/146) | 98.6% (144/146) |

2. Comparison studies:
a. *Method comparison with predicate device:*
The FIDIS™ THYRO assay was compared to the ImmunoWELL™ TPO IgG and TG IgG ELISA assays by testing 247 samples (101 positive for one or both of the antigens, 146 negative samples).  No information about age, gender, and clinical status was available.  Equivocal results were considered negative for the purpose of calculating percent agreements.

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| TPO | ImmunoWELL TPO ELISA IU/mL | | | |
|---|---|---|---|---|
| **FIDIS™ THYRO IU/mL** | **>65** | **45-65** | **<45** | **Total** |
| **>150** | 76 | 9 | 2 | 87 |
| **130-150** | 0 | 3 | 1 | 4 |
| **<130** | 3 | 15 | 138 | 156 |
| **Total** | 79 | 27 | 141 | 247 |

| TG | ImmunoWELL TG ELISA IU/mL | | | |
|---|---|---|---|---|
| **FIDIS™ THYRO IU/mL** | **>120** | **85-120** | **<85** | **Total** |
| **>150** | 40 | 4 | 0 | 44 |
| **130-150** | 0 | 0 | 0 | 0 |
| **<130** | 4 | 21 | 178 | 203 |
| **Total 247** | 44 | 25 | 178 | 247 |

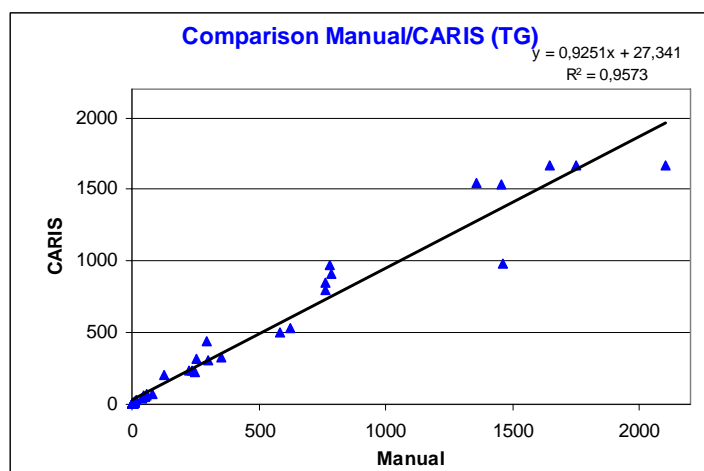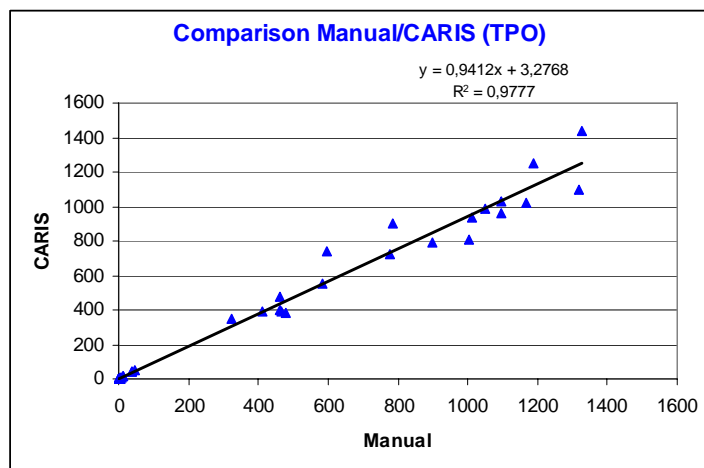| Antigen | Positive percent Agreement (%) | Negative percent Agreement (%) | Overall Agrement (%) |
|---|---|---|---|
| **TPO** | 96.2% (76/79) | 93.5% (157/168) | 94.3% (233/247) |
| **TG** | 90.9% (40/44) | 98.0% (199/203) | 97.6% (239/247) |

<u>Comparison of CARIS and manual method</u>. A comparative study between the CARIS™ Diluting and Dispensing System and the manual technique was performed on 40 samples.

| TG | | Manual FIDIS | |
|---|---|---|---|
| | | Positive | Negative |
| **CARIS™ FIDIS** | Positive | 19 | 1 |
| | Negative | 0 | 20 |

| TPO | | Manual FIDIS | |
|---|---|---|---|
| | | Positive | Negative |
| **CARIS™ FIDIS** | Positive | 20 | 0 |
| | Negative | 0 | 20 |

| Antigenic Specificity | Positive percent Agreement (%) | Negative percent Agreement (%) | Overall Agreement (%) |
|---|---|---|---|
| **TPO** | 100 (20/20) | 100 (20/20) | 100 (40/40) |
| **TG** | 100 (19/19) | 95.24 (20/21) | 97.5 (39/40) |

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Linear Regression analysis of antigenic specificities with CARIS™.

**Comparison Manual/CARIS (TPO)**

$y = 0{,}9412x + 3{,}2768$
$R^2 = 0{,}9777$

**Comparison Manual/CARIS (TG)**

$y = 0{,}9251x + 27{,}341$
$R^2 = 0{,}9573$

The reproducibility of the assay following the optional final wash and delayed analysis was assessed.  Six samples following the overnight wash and delayed analysis were tested 6 times and results were compared to a test that was performed on the same day.  Both sets of test results were similar and are shown below:

| Sample | TPO | | | | TG | | | |
| | A | | B | | A | | B | |
| | mean | CV% | mean | CV% | mean | CV% | mean | CV% |
|---|---|---|---|---|---|---|---|---|
| 1 | 199 | 8 | 183 | 4 | 3 | 16 | 2 | 23 |
| 2 | 239 | 5 | 202 | 3 | 2 | 20 | 2 | 25 |
| 3 | 813 | 8 | 692 | 14 | 63 | 8 | 51 | 11 |
| 4 | 12 | 7 | 10 | 4 | 62 | 2 | 52 | 4 |
| 5 | 42 | 14 | 35 | 10 | 238 | 6 | 211 | 8 |
| 6 | 866 | 11 | 713 | 6 | 303 | 5 | 263 | 7 |

b.  *Matrix comparison:*
    Serum is the only recommended matrix.

3. Clinical studies:
    a. *Clinical Sensitivity:*
       Not available
    b. *Clinical specificity:*
       Not available
    c. *Other clinical supportive data (when a. and b. are not applicable)*:
       Not applicable
4. Clinical cut-off**:**
   Results below 130 IU/mL are considered negative
   Between 130 and 150 IU/mL are considered borderline
   Above 150 IU/mL are considered positive
5. Expected values/Reference range:
   Expected values in the normal population should be negative, however thyroid antibodies may be present in apparently healthy adults.

| Pathology | Anti-TPO | Anti-TG |
|---|---|---|
| Hashimoto | 99% | 85% |
| Thyroid atrophy | 99% | 85% |
| Basedow/Graves | 75% | 50% |
| Healthy adults | 4-8% <br> 15% (*over 60)* | 4-8% <br> 15% (*over 60)* |

**N. Proposed Labeling:**
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.