510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k060264

B. Purpose for Submission:
Clearance of a new device

C. Measurand:
Total Iron

D. Type of Test:
Quantitative

E. Applicant:
Dade Behring, Inc.

F. Proprietary and Established Names:
Dimension® Iron Flex® reagent cartridge (IRON-DF85)

G. Regulatory Information:

1. Regulation section:
   21 CFR § 862.1410
2. Classification:
   Class I, reserved
3. Product code:
   JIY, Iron (non-heme) test system
4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   The IRON method for the Dimension® clinical chemistry system is an in vitro diagnostic test intended to quantitatively measure iron in human serum and plasma.
2. Indication(s) for use:
   The IRON method for the Dimension® clinical chemistry system is an in vitro diagnostic test intended to quantitatively measure iron in human serum and plasma. Iron measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia and other disorders of iron metabolism.
3. Special conditions for use statement(s):
   None
4. Special instrument requirements:
   Dimension® clinical chemistry system

I. Device Description:

The Dimension® IRON Flex® reagent cartridge (DF85) is an in vitro diagnostic device that consists of prepackaged reagents in a plastic eight well cartridge for use on the Dade Behring Dimension® clinical chemistry system for the quantitative determination of iron in serum and plasma.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Dimension® IRN Iron Flex® reagent cartridge (DF49A)
2. Predicate 510(k) number(s):
   k944093, k010061
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative determination of total iron	Quantitative determination of total iron
Reagent Components	Ferene® (chromophore) Thiourea (prevent Cu interference), Ascorbic acid (reducing agent)	Ferene® (chromophore) Thiourea (prevent Cu interference), Ascorbic acid (reducing agent)
Detection	Bi-chromatic endpoint measurement (600 and 700 nm)	Bi-chromatic endpoint measurement (600 and 700 nm)
Assay Methodology	Chromatic	Chromatic
Calibration	Three point linear calibration same Analytical Range 5 to 1,000 µg/dL	Three point linear calibration same Analytical Range 5 to 1,000 µg/dL
Standardization	NIST SRM 937	NIST SRM 937

Differences		
Item	Device	Predicate
Sample Type	Serum or heparinized plasma	Serum only

K. Standard/Guidance Document Referenced (if applicable):

1) Guidance for Industry and FDA Staff - Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use, 11/30/2004
2) Guidance for Industry and FDA Staff; Replacement Reagent and Instrument Family Policy, 12/11/2003
3) Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff, 08/12/2005
4) GP22-A Continuous Quality Improvement Essential Management Approaches
5) ISO 15223 Medical devices – Symbols to be used with medical device labeling

and information to be supplied
6) ISO 14971-2000 Application of risk analysis to Medical devices
7) NCCLS EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods
8) NCCLS EP7-A, Interference Testing in Clinical Chemistry
9) CEN 13640 Stability testing of In-Vitro Diagnostic Devices

L. Test Principle:

Under acidic conditions, iron (Fe^{+++}) bound to the protein transferrin is released. In the presence of the reducing agent ascorbic acid, (Fe^{+++}) is reduced to (Fe^{++}). (Fe^{++}) forms a blue complex with 3-(2-pyridyl)-5,6-bis-2-(5-furyl sulfonic acid)-1,2,4-triazine, disodium salt (Ferene®). The absorbance of the complex, measured using a bichromatic (600, 700 nm) endpoint technique, is directly proportional to the concentration of transferrin-bound iron in the serum.

Fe^{+++} -- Transferrin $\rightarrow$ Fe^{+++} + Transferrin

2 Fe^{+++} + Ascorbic Acid $\rightarrow$ 2 Fe^{++} + Dehydroascorbic Acid + 2 H^{+}

Fe^{++} + 3 Ferene® $\rightarrow$ Fe^{++} -- Ferene®3 complex (absorbs at 600 nm)

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Commercially available Bio-Rad Controls and serum and plasma pools were analyzed in duplicate twice a day for 20 days in accordance with the CLSI/NCCLS EP5-A2 Guidelines. All specific performance characteristic tests were run after normal recommended equipment quality control checks were performed on the Dimension® RxL clinical chemistry system. Five samples were run externally at the Washington University School of Medicine, St Louis, Missouri, using the Dimension® IRON method on the Dimension RxL® clinical chemistry system. These samples are designated in the summary chart below with an (E). Seven samples were run internally at Dade Behring research laboratory facility located in Glasgow, Delaware using the Dimension® IRON method on the Dimension RxL® clinical chemistry system. These samples are designated in the summary chart below with an (I). The following sources of variability existed at each of the testing locations: External Site (E): one instrument, one operator, one reagent lot, one calibration interval, samples analyzed n=1 in two separate cups twice a day for 20 days. Internal Site (I): one instrument, one operator, one reagent lot, one calibration interval, samples analyzed n=1 in two separate cups twice a day for 20 days. The repeatability and within-lab precision coefficients of variation (%CV) were calculated by the analysis of variance method according to the Clinical Laboratory Standards Institute (CLSI) Guideline EP5- A2. The sponsors acceptance criteria for Repeatability %CV is less than or equal to 3% and for Within-lab %CV the

sponsors acceptance criteria less than or equal to 5%. The summary for each of these sites is shown in the table below.

Material	Mean		Standard Deviation (%CV)	
	µg/dL	[µmol/L]	Repeatability	Within-lab
Sample Volume = 40 µL				
Plasma pool (E)	101	18	0.5 [0.09] (0.5)	0.7 [0.13] (0.7)
Serum pool 1 (E)	95	16.9	0.5 [0.09] (0.5)	0.6 [0.11] (0.6)
Serum pool 2 (I)	316	56.6	1.5 [0.27] (0.5)	3.5 [0.63] (1.1)
Serum pool 3 (I)	533	95.4	2.4 [0.43] (0.5)	4.2 [0.75] (0.8)
BioRad Lyphochek® control Level 1 (E)	231	41.3	1.3 [0.23] (0.5)	1.6 [0.29] (0.7)
BioRad Lyphochek® control Level 2 (E)	50	8.9	0.5 [0.09] (1.1)	0.9 [0.16] (1.9)
BioRad Lyphochek® Anemia control Level 1 (E)	26	4.7	0.3 [0.05] (1.3)	0.5 [0.09] (1.9)
Sample Volume = 25 µL				
Serum pool 1 (I)	103	18.5	0.7 [0.13] (0.6)	1.0 [0.18] (0.9)
Serum pool 2 (I)	316	56.6	1.5 [0.27] (0.5)	3.5 [0.63] (1.1)
Serum pool 3 (I)	530	94.9	2.9 [0.52] (0.5)	4.2 [0.75] (0.8)
BioRad Lyphochek® Anemia control Level 1 (I)	32	5.7	0.3 [0.05] (1.3)	0.5 [0.09] (1.9)
BioRad Multiqual® control Level 3 (I)	231	41.3	1.6 [0.29] (0.7)	2.2 [0.39] (0.9)

b. Linearity/assay reportable range:

Linearity was assessed by analyzing solutions of NIST SRM 937. A stock solution of standard reference material was prepared according to the directions specified by NIST. Weighed amounts of SRM937 metal were dissolved in

hydrochloric acid then diluted with deionized water to a concentration of 5190 µg/dL. Test concentrations were prepared by dilution of this stock and then sequential mixing to create equally spaced samples ranging from 0 to 2000 µg/dL iron. The linearity of iron on the Dimension® RxL by the Dimension® IRON assay was evaluated by comparing observed versus expected values across the expected range. A linear regression analysis was performed on the data and plotted. The observed linearity (using least squares regression analysis) gave the following: Observed = 0.999(Expected) + 0.178; r = 0.9999 from 0 to 2000 µg/dL.

Sample	Expected Value, µg/dL	Observed Value, µg/dL
1	0	-3
2	250	248
3	500	503
4	750	753
5	1000	1003
6	1250	1251
7	1500	1499
8	1750	1747
9	2000	2000

The assay range claim is 5 to 1000 µg/dL. The sponsor indicates that values detected as >1000 µg/dL should be manually diluted according to the package insert to obtain results within assay range.

To ascertain device performance over the clinically relevant levels between 5 to 250 µg/dL, data with values in this range from the method comparison study were used (n=107). The observed least squares regression statistics were the following: Observed = 1.003(Expected) – 2.64; r = 0.9986 from 9 to 251 µg/dL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Calibrator set points are traceable to NIST SRM 937. The calibrator was approved under k060264 as a kit component required but not provided. The shelf-life of the calibrator is 12 months.

d. Detection limit:
The analytical sensitivity (limit of the blank) represents the lowest concentration of iron that can be distinguished from zero. This analytical sensitivity was defined as the mean value (n=20) plus two standard deviations of the Level 1 (0mg/L) IRON Calibrator. The Level 1(0mg/L) IRON calibrator was run n=20 on one Dimension® RxL clinical chemistry system using one reagent lot on one instrument at both the 40uL and 25 uL sample size. The analytical sensitivity of the IRON method is stated as 5 µg/dL using a 40 µL sample size and 5 µg/dL using a 25 µL sample size.

e. Analytical specificity:

Dimension® IRON was evaluated for interference according to CLSI/NCCLS EP7-A. Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. The sponsor considered bias exceeding 10% to be interference. Iron dextran at a concentration of 60 ug/mL increases the IRON result by 63 µg/dL at an IRON concentration of 36 µg/dL [and by 69 µg/dL at an IRON concentration of 131 µg/dL. The following substances do not interfere with the IRON method when present in serum in the amounts indicated. Systematic inaccuracies (bias) due to these substances are less than 10% at an iron concentration of 26 to 38 µg/dL and 118 to 136 µg/dL.

Compound	Concentration [SI Units]	Compound	Concentration [SI Units]
Acarbose	180 µg/mL [200 µmol/L]	Heparin	8 U/mL [8000 U/L]
Acetaminophen	20 mg/dL [1323 µmol/L]	Hydrochlorothiazide	5.9 µg/mL [198 µmol/L]
Allopurinol	40 µg/dL [253 µmol/L]	Ibuprofen	50 mg/dL [2425 µmol/L]
Amikacin	15 mg/dL [256 µmol/L]	Immunoglobulin G (IgG)	5 g/dL [50 g/L]
Amiodorone HCl	6 µg/mL [8.8 µmol/L]	Insulin	0.018 u/mL [18 U/L]
Ampicillin	5.3 mg/dL [152 µmol/L]	Isosorbide dinitrate	150 ng/mL [635 µmol/L]
Ascorbic Acid	5 mg/dL [227 µmol/L]	Lidocaine	6 mg/dL [256 µmol/L]
Atenolol	1 mg/dL [37.5 µmol/L]	Lithium	3.5 mg/dL [5043 µmol/L]
Atorvastatin	600 µg/L [0.4 umol/L]	Losartan potassium	10 mg/dL [0.22 mmol/L]
Caffeine	10 mg/dL [515 µmol/L]	Magnesium	15 mg/dL [1.6 mmol/L]
Calcitriol	0.3 µg/mL [0.7 µmol/L]	Metformin	40 µg/mL [241 µmol/L]
Calcium	15 mg/dL [1.4 mmol/L]	Nateglinide	72 µg/mL [227 µmol/L]
Captopril	22 µg/mL [101 µmol/L]	Niacin	1.2 mg/mL [9.7 mmol/L]
Carbamazepine	12 mg/dL [508 µmol/L]	Nicotine	2 mg/dL [123 µmol/L]
Chloramphenicol	25 mg/dL [774 µmol/L]	Nitrofurantoin	4 µg/mL [17 µmol/L]
Chlordiazepoxide	2 mg/dL [66 µmol/L]	Nortryptiline	1 µg/mL [3 µmol/L]
Chlorpromazine	5 mg/dL [157 µmol/L]	Paricalcitol	8.4 ng/mL [20.2 nmol/L]
Cholesterol	500 mg/dL [12.9 mmol/L]	Penicillin G	25 U/mL [25,000 U/L]
Cinacalcet hydrochloride	18 ng/mL [46 nmol/L]	Pentobarbital	10 mg/dL [442 µmol/L]
Cimetidine	10 mg/dL [396 µmol/L]	Phenobarbital	15 mg/dL [646 µmol/L]
Copper	300 µg/dL [22.3 µmol/L]	Phenytoin	10 mg/dL [396 µmol/L]
Creatinine	30 mg/dL [2652 µmol/L]	Primidone	10 mg/dL [458 µmol/L]
Deferoxamine	250 ng/dL [3.8 nmol/L]	Propoxyphene	0.4 mg/dL [11.8 µmol/L]
Dextran 40	6000 mg/dL [1500 µmol/L]	Protein: Albumin	6 g/dL [60 g/L]
Diazepam	2 mg/dL [70 µmol/L]	Protein: Total	12 g/dL [120 g/L]
Diltiazem hydrochloride	40 ng/mL [89 nmol/L]	Pyridoxine	6 ng/mL [29 nmol/L]

Compound	Concentration [SI Units]	Compound	Concentration [SI Units]
Digoxin	5 ng/mL [6.2 nmol/L]	Repaglinide	7.2 mg/mL [15.9 mmol/L]
Disopyramide phosphate	4 mg/dL [91.8 µmol/L]	Rheumatoid Factor	500 U/mL [500 mIU/L]
Epoetin alfa	456 mU/mL [456 U/L]	Rosiglitazone maleate	48 µg/dL [101 µmol/L]
Erythromycin	20 mg/dL [273 µmol/L]	Salicylic Acid	60 mg/dL [4.34 mmol/L]
Ethanol	400 mg/dL [87 mmol/L]	Streptokinase	300 IU/mL [300 IU/mL]
Ethosuximide	30 mg/dL [2125 µmol/L]	Sulfamethoxazole	1 mg/dL [39 µmol/L]
Fenofibrate	45 µg/dL [125 µmol/L]	Theophylline	4 mg/dL [222 µmol/L]
Ferritin	200 ng/mL [449 pmol/L]	Trimethoprim	0.2 mg/dL [6.9 µmol/L]
Folic acid	1 ng/mL [2.3 nmol/L]	Triamterene	9 µg/mL [36 µmol/L]
Fluvastatin	48 mg/dL [1.1 mmol/L]	Urea	500 mg/dL [83 mmol/L]
Furosemide	6 mg/dL [181 µmol/L]	Uric Acid	20 mg/dL [1190 µmol/L]
Gemfibrozile	75 µg/mL [299 µmol/L]	Valproic Acid	50 mg/dL [3467 µmol/L]
Gentamicin	12 mg/dL [251 µmol/L]	Warfarin sodium	40 µg/mL [121 µmol/L]
Glyburide	2 µg/mL [4 µmol/L]		

The IRON method (using the standard sample size of 40 uL) was evaluated for interference according to CLSI/NCCLS EP7-A. The sponsor defined the bias as the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. The sponsor considered bias exceeding 10% to be interference.

Substance Tested	Test Concentration [SI units]	IRON Concentration [SI Units]	Bias %
Hemoglobin (hemolysate)	50 mg/dL [0.03 mmol/L] (monomer) 200 mg/dL [0.12 mmol/L] (monomer)	107 µg/dL [19.3 µmol/L]	<10% +10%
Bilirubin (unconjugated)	80 mg/dL [1368 µmol/L]	107 µg/dL [19.3 µmol/L]	<10%
Lipemia (Intralipid®)	1000 mg/dL [11.43 mmol/L] 3000mg/dL [34.29 mmol/L]	107 µg/dL [19.3 µmol/L]	<10% +71%

The IRON method (using the reduced sample size of 25 uL) was evaluated for interference according to CLSI/NCCLS EP7-A11. The sponsor defined the bias as the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. The sponsor considered bias exceeding 10% to be interference.

Substance Tested	Test Concentration [SI units]	IRON Concentration [SI Units]	Bias %
Hemoglobin	50 mg/dL [0.03 mmol/L]	55µg/dL [9.8 µmol/L]	<10%

Substance Tested	Test Concentration [SI units]	IRON Concentration [SI Units]	Bias %
(hemolysate)	(monomer) 200 mg/dL [0.12 mmol/L] (monomer)	55µg/dL [9.8 µmol/L]	+22%
Bilirubin (unconjugated)	80 mg/dL [1368 µmol/L]	53 µg/dL [9.5µmol/L]	<10%
Lipemia (Intralipid®)	3000mg/dL [34.29 mmol/L]	27 µg/dL [4.8µmol/L]	<10%

f. Assay cut-off:
Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:
A total of 147 serum samples were run using the IRN and IRON methods on the Dimension® RxL clinical chemistry system. Ninety-nine (99) individual native serum patient samples were tested. Due to the difficulty to obtain native individual samples containing iron concentrations that adequately span the entire assay range of the Dimension IRON assay, some spiked samples were added to the method comparison study. 48 different individual serum samples were spiked with various amounts of NIST-937 reference iron material.

The distribution of samples included 10 visibly icteric and 10 visibly lipemic samples. Samples containing hemoglobin have been identified as a known interferent for the Dimension® IRON method and were not included in this study. This limitation is described in the "Specimen Collection" section of the package insert. The following represents the sample breakdown across the assay range.

Range of Iron Concentration	Number of Specimens Tested
<50 µg/dL	41
50-150 µg/dL	51
150-300 µg/dL	18
300-1000 µg/dL	37
Total	147

Linear regression analysis gave the following relationship:
Device = 0.980(Predicate) – 0.488; r = 0.9996

b. Matrix comparison:

One hundred and twenty nine (129) matched sets of serum and plasma specimens

were analyzed to estimate bias between serum and either lithium or sodium heparin plasma sample types.

Serum and heparinized (lithium or sodium heparin) plasma are the recommended specimens for the Dimension® Automated iron (IRON) assay (DF85). In order to compare specimen types with analyte values that span the assay range, fifty (50) of the samples were spiked with varying amounts of $FeCl_2$.

No clinically significant difference was observed between serum and plasma samples. Results are summarized in the table below.

Comparison	Slope	Y-Intercept	*r*
Serum vs Na heparin plasma	0.988	0.804	0.999
Serum vs Li heparin plasma	0.985	1.42	0.999
Li heparin vs Na heparin plasma	1.00	-0.590	0.9997

To determine the effect of freezing and thawing on iron in 20 serum and 20 lithium hepranized plasma samples, samples were run fresh, in duplicate, on the Dimension® RxL clinical chemical system. The samples were frozen, thawed and then run in duplicate on the Dimension® RxL clinical chemical system. A bias was calculated by subtracting the mean of the frozen results from the mean of fresh results. The average bias for both serum and lithium heparinized samples was -2. No statistical difference was observed for either serum or plasma.

3. Clinical studies:

   a. Clinical Sensitivity:
   Not applicable.

   b. Clinical specificity:
   Not applicable.

   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off:
   Not applicable.

5. Expected values/Reference range:
   The reference range for total iron is indicated to be:

   Males: 65-175 µg/dL [11.6-31.3 µmol/L]
   Females: 50-170 µg/dL [9.0- 30.4 µmol/L]

   These values were quoted from the following reference:
   Kaplan LA, Psece AJ. Clinical Chemistry Theory, Analysis, and Correlation, 3rd

ed. St. Louis; Mosby, Inc., 1996: p 699, 713-714.

Normal reference intervals can differ by as much as 35% between commercial iron methods, therefore the sponsor advises customers that each laboratory establish its own expected values for iron as performed on the Dimension® system.

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.