

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k051110

B. Purpose for Submission:

New device

C. Analyte:

1,25-Dihydroxy Vitamin D

D. Type of Test:

Enzymeimmunoassay

E. Applicant:

Immunodiagnostic Systems, Ltd.

F. Proprietary and Established Names:

IDS 1,25-Dihydroxy Vitamin D EIA

G. Regulatory Information:

1. Regulation section:
21 CFR § 862.1825, Vitamin D test system
21 CFR § 862.1660, Quality control material
2. Classification:
Class II
3. Product Code:
MRG, Vitamin D test system
JJX, Single analyte controls
4. Panel:
Chemistry (75)

H. Intended Use:

1. Intended use(s):
See Indications for Use
2. Indication(s) for use:
The IDS 1,25-Dihydroxy Vitamin D kit is a complete assay system intended for purification of 1,25-dihydroxyvitamin D (1,25D) in human serum or

plasma by immunoextraction followed by quantitation by enzymeimmunoassay. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of 1,25D deficiency associated with renal disease in adult populations.

3. Special condition for use statement(s):
For in vitro diagnostic use only. Results are to be used in conjunction with other clinical and laboratory data.
4. Special instrument Requirements:
Instrumentation common to most high complexity laboratories: centrifuge, sample mixer, vortex, photometric microplate reader and data analysis equipment

I. Device Description:

The IDS 1,5-Dihydroxy Vitamin D EIA kit is a complete assay system of the purification of 1,25D in patient samples by immunoextraction followed by quantitation by EIA. Patient samples are diluted and 1,25D extracted from potential cross-reactants by incubation for 90 minutes with a highly specific solid phase monoclonal anti-1,25D. The immunoextraction gel is then washed and purified 1,25D eluted directly into glass assay tubes. Reconstituted eluates and calibrators are incubated overnight with a highly specific sheep anti-1,25D. Then a portion of this is incubated for 90 minutes with shaking in microplate wells which are coated with a specific anti-sheep antibody. 1,25D linked to biotin is then added and the plate shaken for a further 60 minutes before aspiration and washing. Enzyme (horse peroxidase) labelled avidin is added and binds selectively to complexed biotin and, following a further wash step, color is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtiter plate reader, colour intensity developed being inversely proportional to the concentration of 1, 25D.

Controls included in the device are prepared from human source materials. They have tested negative by FDA recommended assays for the presence of antibodies to HIV (I and II), to HCV, and to Hepatitis B surface antigen.

J. Substantial Equivalence Information:

1. Predicate device name(s):
GAMMA-B 1,25-Dihydroxy Vitamin D RIA
2. Predicate K number(s):
k042519

3. Comparison with predicate:

| Similarities | | |
|------------------------|--|---|
| Item | Device | Predicate |
| Intended Use | Quantitative determination of 1,25D for assessment of deficiency associated with renal disease | Quantitative determination of 1,25-D for assessment of deficiency associated with renal disease |
| Test Principle | Immunoassay – Competitive binding technique | Immunoassay – Competitive binding technique |
| Antibody Format | Liquid Phase: Polyclonal anti-1,25-Dihydroxyvitamin D [Sheep] in an aqueous buffer | Liquid Phase: Polyclonal anti-1,25-Dihydroxyvitamin D [Sheep] in an aqueous buffer |
| Antigen in calibrators | 1,25- DihydroxyvitaminD ₃ | 1,25-Dihydroxyvitamin D ₃ |
| Sample type | Serum or plasma (EDTA or Heparin) | Serum or plasma (EDTA or Heparin) |
| Sample Volume | 100 µL | 100 µL |
| Differences | | |
| Item | Device | Predicate |
| Detection System | Colorimetric | Isotope |
| Test Method | Enzymeimmunoassay (EIA) | Radioimmunoassay (RIA) |

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

CLSI C28-A2 – How to Define and Determine Reference Intervals in the Clinical Laboratory

CLSI EP9-A – Method Comparison and Bias Estimation Using Patient Samples

CLSI EP7-A – Interference Testing in Clinical Chemistry

CLSI EP5-A – Evaluation of Precision Performance of Clinical Chemistry Devices

CLSI EP6-A – Evaluation of the Linearity of Quantitative Analytical Methods

L. Test Principle:

Enzymeimmunoassay

M. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

Precision was evaluated in accordance with CLSI EP-5A Evaluation of Precision Performance of Clinical Chemistry Devices. Three human serum controls were assayed over 17 assay days spanning more than 49 operating days. The assays were performed by multiple operators using multiple reagents lots.

| Control | n | Mean (pmol/L) | Within-run | | Total | |
|---------|----|------------------|------------|------|-------|------|
| | | | SD | CV% | SD | CV% |
| 1 | 28 | 19.0 | 2.0 | 10.7 | 3.8 | 19.7 |
| 2 | 28 | 53.2 | 5.6 | 10.5 | 9.1 | 17.1 |
| 3 | 28 | 152 | 14.1 | 9.3 | 26.7 | 17.6 |

Recovery was assayed by adding 1,25D (D₃) to samples prior to extraction and assay. Results are summarized below (1,25D concentrations are in pmol/L).

| Sample Concentration | 1,25D (D ₃) added | Measured pmol/L | Recovery pmol/L | Recovery % |
|----------------------|-------------------------------|-----------------|-----------------|------------|
| 62.7 | 46.5 | 106.4 | 43.6 | 98 % |
| 62.7 | 93.0 | 140.7 | 78.0 | 84 % |
| 46.2 | 54.4 | 100.6 | 54.4 | 100% |
| 46.2 | 108.8 | 161.3 | 115.1 | 106 % |
| Mean | | | | 96% |

b. Linearity/assay reportable range:

Linearity studies were designed using CLSI EP6A, Evaluation of the Linearity of Quantitative Analytical Methods. Samples containing varying concentrations of 1,25D prior were assayed in duplicate. The resulting mean concentrations were compared to predicated concentrations. Samples were prepared by diluting a patient sample with assay buffer prior to extraction and assay. The reportable range was determined to be <6 – 333 pmol/L.

c. Traceability (controls, calibrators, or method):

Calibrators and controls are identical to those in the predicate device Gamma-B 1,25-Dihydroxy Vitamin D kit. (K042519). Calibrators are assigned values against primary reference calibrators in the IDS 1,25-Dihydroxy Vitamin D EIA. The mean potency for each calibrator level is calculated from the results of at least 9 independent assays. The assays are carried out on different days by different operators using a variety of reagent batches and expiries.

Acceptable ranges for the controls are assigned by assaying the controls with the IDS 1,25-Dihydroxy Vitamin D EIA. A minimum of 20 values are obtained by performing the assay on different days, by different operators using a variety of reagent batches. The value claimed is the mean of the measurements and the acceptable range is given as the mean \pm 24%.

Expiration date of 12 months for the calibrators and 18 months for the controls when stored at 4 °C is based on real time studies that

show no or minimal deterioration of the calibrators up to 17 months of storage and up to 25 months for the controls. Stability of calibrators and controls after they have been reconstituted and stored at -20 °C, including several freeze thaw cycles, has been established past the recommended 8 week expiry.

d. Detection limit:

The analytical sensitivity of this device was determined to be 11 pmol/L is defined as the concentration corresponding to the mean counts from 10 measurements of the zero calibrator minus 2 standard deviations.

e. Analytical specificity:

Specificity of the antibodies was tested against 2 common metabolites of Vitamin D as well as against the intended analytes. The specificity of the kit was assessed with the following analytes at 50% binding of the zero calibrator.

| Molecule | Cross-reactivity |
|---|------------------|
| 1,25-Dihydroxyvitamin (D ₃) | 100.0 % |
| 1,25-Dihydroxyvitamin D ₂ | 39 % |
| 24,25-Dihydroxyvitamin D ₃ | 0.058 % |
| 25-Hydroxyvitamin D ₃ | 0.009 % |

Interference testing was conducted in accordance with CLSI EP7-A. Common endogenous substances – haemoglobin, bilirubin, lipid and urea were tested. No interference was found at the levels tested.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The *IDS 1,25-Dihydroxy Vitamin D EIA* kit was compared against a recognized radioimmunoassay for the quantitative determination of 1,25-dihydroxyvitamin D following CLSI EP9-A2, “Method Comparison and Bias Estimation Using Patient Samples”. A population of 152 samples, selected to represent a wide range of 1,25-dihydroxyvitamin D [10-402 pmol/L], were assayed by each method. Passing & Bablok regression analysis was performed on the comparative data with the following result:

$IDS = 0.94X + 7.2$ (95% confidence intervals of the slope and intercept were 0.89 to 1.01, and 2.1 to 12.7 respectively);

Correlation coefficient (r) = 0.95.

b. Matrix comparison:

A study comparing serum to plasma (EDTA & Heparin) for IDS 1,25-Dihydrox Vitamin D EIA was performed to establish matrix equivalency. No statistical difference in the means was observed between different sample types.

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 95% reference interval for Normal Adults was determined from 120 apparently healthy adults of US origin, was calculated by a nonparametric method following CLSI guideline C28-A2, "How to Define and Determine Reference Intervals in the Clinical Laboratory".

Normal Adults 39 – 193 pmol/L (n=120)

End-stage of renal disease <6 – 22 pmol/L (n = 24)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirement of 21 CFR part 809.10

O. Conclusion:

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

