**TITLE: Intact PTH Reagent Pack**

#  Intact PTH Calibrators

**PRINCIPLE:**

The VITROS Intact PTH test is performed using the VITROS Intact PTH Reagent Pack and the VITROS Intact PTH Calibrators on the VITROS 5600 Integrated

System using Intellicheck® Technology. An immunometric immunoassay technique is used, which involves the simultaneous reaction of PTH present in the sample with a biotinylated antibody (goat polyclonal anti‑PTH39-84) and a horseradish

peroxidase (HRP)-labeled antibody conjugate (goat polyclonal anti-PTH1-34). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of PTH present.

# Reaction Scheme

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## Warnings and Precautions

WARNING: Potentially Infectious Material

Use caution when handling material of human origin. Consider all samples potentially

infectious. No test method can offer complete assurance that hepatitis B virus,

hepatitis C virus (HCV), human immunodeficiency virus (HIV 1+2) or other infectious

agents are absent. Handle, use, store and dispose of solid and liquid waste from

samples and test components, in accordance with procedures defined by appropriate

national biohazard safety guideline or regulation (e.g. CLSI document M29).5

WARNING: Contains Proclin 300 (CAS 55965-84-9) and Proclin 950 (CAS 2682-20-4)

The VITROS Intact PTH Reagent Pack contains Proclin 950 and the VITROS Intact

PTH calibrators and Range Verifiers contain Proclin 300. R43: May cause

sensitization by skin contact. S24: Avoid contact with skin. S37: Wear suitable gloves.

**CLINICAL SIGNIFICANCE:**

Parathyroid hormone (PTH) is a single chain 84 amino acid polypeptide produced by the parathyroid gland. After PTH is secreted into the blood stream it undergoes extensive proteolysis to generate various fragments. In contrast to its degradation products, the concentration of Intact PTH is relatively independent of glomerular filtration rate and reflects the biologically active portion of the hormone.

The primary role of PTH is to maintain calcium homeostasis via its interaction with calcitonin. PTH measurement is an important aid in the diagnosis of disorders of calcium metabolism. PTH synthesis and secretion are triggered rapidly by low concentrations of ionized calcium (Cai). The biological activities of PTH are to increase absorption of dietary calcium, decrease renal clearance and mobilize skeletal calcium stores. Abnormally high Cai concentrations suppress secretion of PTH. In conjunction with serum calcium levels, the PTH assay may be used as an aid in the differential diagnosis of hypercalcemia, hypocalcemia and parathyroid disorders. PTH determination is important in monitoring dialysis patients to manage renal osteodystrophy.

Guidelines from the National Academy of Clinical Biochemistry recommend the use of intraoperative parathyroid hormone testing for patients during surgery for hyperparathyroidism, especially in minimally invasive or directed procedures, as well as

for patients undergoing reoperation. For patients undergoing parathyroidectomy it is recommended that preoperative and pre‑excision samples are taken. Samples should also be drawn at 5 and 10 minutes post resection and a >50% reduction in PTH levels from the highest baseline may be used as criteria for surgical success.

**SPECIMEN COLLECTION:**

## Patient Preparation

No special patient preparation is necessary.

## Specimens Recommended

• Serum

• Heparin plasma

## Specimens Not Recommended

• Do not use turbid specimens. Turbidity in specimens may affect test results.

• Do not use hemolyzed specimens.

## Special Precautions

IMPORTANT: Intact PTH is labile and susceptible to fragmentation. Correct handling of patient samples is necessary to ensure that the PTH molecule remains intact. The degree

of fragmentation will depend on both time and temperature of storage.

Specimen types should not be used interchangeably during the serial monitoring of

an individual patient as measured concentrations may vary slightly between sample

types.

Certain collection devices have been reported to affect other analytes and tests.

Owing to the variety of specimen collection devices available, Ortho Clinical

Diagnostics is unable to provide a definitive statement on the performance of its

products with these devices. Confirm that your collection devices are compatible with

this test. Storage of samples collected and stored in serum separator tubes for more

than one day may result in a decrease in concentration of up to 20%.

## Specimen Collection and Preparation

• Collect specimens using standard procedures.

• Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.

• Thoroughly mix samples by inversion and bring to 15–30 °C (59–86 °F) before use.

• The VITROS Intact PTH test uses 80 μL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

* Prior to Parathyroid surgery an Intact PTH is performed. Ten to twenty minutes after the Parathyroid gland is removed another Intact PTH should be ordered and performed. When completed the result for the second Intact PTH should be called to surgery.

## Handling and Storage Conditions

• Handle samples in stoppered containers to avoid contamination and evaporation.

• The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. Refer to the operating instructions for your system.

• Return to 2–8 °C (36–46 °F) as soon as possible after use, or load sufficient volume for a single determination.

• Serum and plasma samples may be stored for up to 2 days at 2–8 °C (36–46 °F) or 4 weeks at -20 °C (-4 °F).

• Avoid repeated freeze‑thaw cycles.

**REAGENTS:**

# Reagent Pack Contents

1 reagent pack containing:

• 100 coated wells (streptavidin, binds ≥3 ng biotin/well)

• 6.2 mL biotinylated antibody reagent (biotin-goat polyclonal anti-PTH, binds ≥20,000 pg PTH/mL) in buffer with bovine gamma globulin, bovine serum albumin, and antimicrobial agent

• 8.4 mL conjugate reagent (HRP-goat polyclonal anti-PTH, binds ≥8000 pg PTH/mL) in buffer with bovine serum albumin and antimicrobial agent

# Reagent Pack Handling

• The reagent pack is supplied ready for use.

• The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading on the system.

• As with all immunoassay protein-based solutions, inappropriate handling of the reagent pack can cause foam to occur on the surface of the reagent. Avoid agitation, which may cause foaming or the formation of bubbles.

– If reagent packs are dropped or agitated, small levels of fine foam could be generated that may not be detected by the system.

– Reagent packs containing fine foam that is not detected by the system, may show a negative bias.

• If you must use a dropped or agitated reagent pack before it has been allowed to settle, you should verify performance by running high and low quality control samples after loading the pack on the system.

# Reagent Pack Storage and Preparation

Reagent Storage Condition Stability

**Reagent Storage Condition Stability**

Unopened Refrigerated 2–8 °C expiration date

Opened On system ≤8 weeks

• The VITROS Intact PTH Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.

• Do not freeze unopened reagent packs.

• Load reagent packs directly from refrigerated storage to minimize condensation.

• Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.

# Calibrator Contents

• 3 sets of VITROS Intact PTH Calibrators 1, 2 and 3 (freeze-dried, synthetic PTH in buffer with bovine serum albumin and antimicrobial agent, reconstitution volume 1.0 mL); nominal values 0; 100 and 1500 pg/mL (0; 10.6 and 159 pmol/L)

• 24 calibrator bar code labels (8 for each calibrator)

# Calibrator Handling

• Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30 °C (59–86 °F) before use. Each pack contains sufficient for a minimum of 6 determinations of each calibrator.

• Handle calibrators in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8 °C as soon as possible after use, or load only sufficient for a single determination.

**Calibrator Storage and Preparation**

# Calibrator Storage Condition Stability

Unopened Refrigerated 2–8 °C expiration date

Opened Refrigerated 2–8 °C ≤1 day

Opened Frozen -20 °C ≤13 weeks

• VITROS Intact PTH Calibrators are supplied freeze-dried.

• VITROS Intact PTH Calibrators are suitable for use until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

• Reconstitute with 1.0 mL distilled water.

• Opened, reconstituted calibrators may be stored frozen (with no more than 1 freeze-thaw cycle).

• The VITROS Intact PTH test uses 80 μL of calibrator for each determination. Transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

# Calibration Procedure

• Calibration is lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.

• A Master Calibration (a dose response curve covering the full calibration range) is established for each new reagent lot. Concentrations for the linked lot of calibrators are determined from the Master Calibration.

• Ensure that the Master Calibration for each new reagent lot is available on your system.

• Process calibrators in the same manner as samples. Calibration need not be programmed if bar code labels are used; load the calibrators in any order, calibration will be initiated automatically.

• When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration curve is assessed against a range of quality parameters, and if acceptable, it is stored for use with any reagent pack of that lot.

• The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.

• Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.

• Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration refer to the operating instructions for your system.

Refer to the operating instructions for your system for detailed instructions on the calibration process.

# When to Calibrate

• Calibrate when the reagent pack and calibrator lot changes.

• Calibrate every 28 days.

• After specified service procedures have been performed.

• If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

# Traceability of Calibration

Calibration of the VITROS Intact PTH test is traceable to in-house reference calibrators, which have been value assigned to correlate to another commercially available test.

# Calibration Model

A modified four‑parameter logistic curve fit function is used to construct the Master Calibration. The calibration process rescales the Master Calibration to establish a valid stored curve for the VITROS Immunodiagnostic System and VITROS Integrated

Systems.

**QUALITY CONTROL:**

Refer to Chemistry Quality Control Procedure for Bio-Rad Controls.

# Quality Control Material Selection

VITROS Intact PTH Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems.

VITROS Intact PTH Controls contain 3 levels of PTH (low, medium and high). The performance of other commercial control fluids should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other Intact PTH methods if they contain high concentrations of preservatives, stabilizers, or other nonphysiological additives, or otherwise depart from a true sample matrix.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Intact PTH test.

# Quality Control Procedure Recommendations

• Good laboratory practice requires that controls be processed to verify the performance of the test.

• Choose control levels that check the clinically relevant concentrations.

• To verify system performance, analyze control materials:

After calibration

According to local regulations or at least once each day that the test is being performed

After specified service procedures are performed

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

Analyze quality control materials in the same manner as patient specimens.

• If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.

• Refer to published guidelines for general quality control recommendations.

For more detailed information, refer to the operating instructions for your system.

## Quality Control Material Preparation and Storage

Refer to the manufacturer’s product literature for preparation, storage, and stability information.

**PROCEDURE:**

## Materials Provided

• VITROS Immunodiagnostic Products Intact PTH Reagent Pack

• VITROS Immunodiagnostic Products Intact PTH Calibrators

## Materials Required but Not Provided

• VITROS Immunodiagnostic Products Signal Reagent

• VITROS Immunodiagnostic Products Universal Wash Reagent

• Quality control materials such as VITROS Immunodiagnostic Products Intact PTH Controls

• VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

## Operating Instructions

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.

For detailed information refer to the operating instructions for your system.

Note: Do not use visibly damaged product.

## Sample Dilution

Intact PTH concentrations above the measuring range should be reported as >5000 pg/mL The dilution of samples in the VITROS Intact PTH test is not supported.

## Default Test Name

The default test name which will appear on patient reports is Intact PTH. The default short name that will appear on the test selection menus and laboratory reports is iPTH. These defaults may be reconfigured, if required. For detailed information refer to the operating instructions for your system.

**Measuring (Reportable) Range -** 3.4–5000 pg/mL

**RESULTS**

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

**Reporting Units** - pg/mL

**Reference Interval** - 7.5–53.5 pg/mL

This reference interval is the central 95% of results of a study of 240 patients with normal calcium, TSH, creatinine and Vitamin D values.

**PROCEDURE NOTES:**

## Limitations of the Procedure

## Known Interferences

The VITROS Intact PTH test was evaluated for interference consistent with CLSI document EP7. Commonly encountered substances were tested on 2 lots of reagents. Of the compounds tested, hemoglobin may interfere with the VITROS iPTH test

resulting in a positive bias as shown in the table below.

Refer to “Specificity” for a list of compounds tested that did not show interference.

**Interferent Interferent Concentration Units = pg/mL Analyte Conc Bias**

Hemoglobin 0.155 mmol/L/250 mg/dL 44.2 pg/mL 4.1 pg/mL

## Other Limitations

• The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.

• Biotin levels in serum remain elevated for up to 24 hours after oral or intravenous biotin administration.

• Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results which are inconsistent with clinical observations indicate the need for additional testing.

• Certain drugs and clinical conditions are known to alter PTH concentrations in vivo. For additional information, refer to one of the published summaries.

• The VITROS Intact PTH test has no high dose hook effect up to 1,218,400 pg/mL .

• Do not use quality control materials preserved with azide.

# Performance Characteristics

 **Limit of Detection**

The Limit of Detection (LoD) for VITROS Intact PTH is 2.8 pg/mL , determined consistent with NCCLS documentEP17 and with proportions of false positives (α) less than 1% and false negatives (β) less than 1%; based on 575 determinations, with 1 blank and 5 low-level samples. The Limit of Blank (LoB) is 1.2 pg/mL . The Limit of Quantitation (LoQ) is 3.4 pg/mL as determined by the lowest concentration at which precision and accuracy design requirements are still met and within the linear range of the test.

##  Limit of Blank, Limit of Detection Limit of Quantitation

 LoB\* LoD\*\* LoQ\*\*\*

 1.2 pg/mL 2.8 pg/mL 3.4 pg/mL

\* Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term "analytical sensitivity."

\*\* Proportions of false positives (α) and false negatives (β) were less than 1%; based on 575 determinations, with 1 blank and 5 low-levelsamples.

\*\*\* The level of imprecision used to accept the LoQ was within 25%.

**Accuracy (Method Comparison)**

Accuracy was evaluated consistent with NCCLS document EP9. The table shows the results of a method comparison study using serum samples from a variety of clinical categories analyzed on the VITROS Immunodiagnostic and Integrated Systems compared with those analyzed using the Roche Elecsys PTH test. The relationship between the 2 methods was determined by Passing and Bablok regression.

The table also shows the results of method comparison studies using serum and plasma samples from a variety of clinical categories analyzed on the VITROS 5600 Integrated System compared with those analyzed using the VITROS 3600 Immunodiagnostic System. The relationship between the 2 methods was determined by Passing and Bablok regression.

**Samples across the Measuring Range**

System n Slope CorrelationCoefficient Range ofSamples Intercept

5600 vs. Comparative Method 412 1.01 0.99 3.5–4556 +0.54 3600 vs. 5600 445 1.03 1.00 3.4–4556 -0.02

Samples below 100 pg/mL

System n Slope CorrelationCoefficient Range ofSamples Intercept

5600 vs. Comparative Method 325 1.00 0.92 3.5–97.5 +0.74 3600 vs. 5600 334 1.05 0.99 3.4–95.7 -0.36

**Precision**

VITROS 5600 Integrated System

Precision was evaluated consistent with NCCLS document EP5. Two replicates of each of 3 freeze-dried control samples and 6 patient sample pools were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 1 reagent lot on each system. The data presented are a representation of the product performance.

\* Within-run (repeatability). Between Duplicate precision averaged over all runs

\*\* Within-calibration. Total precision with weighted components of within-run, between–run and between-day variation.

\*\*\* Within-lab. A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 4 calibrations

**Specificity**

Substances that do not Interfere

The VITROS Intact PTH test was evaluated for interference consistent with CLSI document EP7. Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at PTH concentrations of 14.2–48.0 pg/mL .

 Compound Concentration

 Azide 20 mg/dL

 Bilirubin 20 mg/dL

 Biotin 500 ng/dL 20.4 nmol/L

 Dipyrone 100 mg/dL 3 mmol/L

 Hemoglobin (Hemolysate)\* 161 mg/dL 0.100 mmol/L

 Intralipid 850 mg/dL 8.5 g/L

 Triolein 2500 mg/dL

\* Hemolysate was added to a series of specimens with VITROS Intact PTH concentration of 36.8–43.8 pg/mL (3.9–4.6 pmol/L).

No interference is seen with Rheumatoid Factor (RF concentration up to 4935 IU/mL) or Human Anti Mouse Antibody (HAMA concentration up to 1825 ng/mL) or with HAMA spiked endogenous samples (HAMA concentration up to 2000 ng/mL).

**Cross-Reactivity**

The cross-reactivity of the VITROS Intact PTH test was evaluated by adding the following substances to samples containing no analyte.

 Mean PTH of

 Cross-reactant pool

Cross-reactant Tested Concentration pg/mL %Cross-reactivity

Bone Specific alkaline phosphatase 7.5 ng/mL <3.4 NA\*

Calcitonin 10,000 pg/mL <3.4 NA\*

ß-Cross laps 1 ng/mL <3.4 NA\*

Osteocalcin 50 ng/mL <3.4 NA\*

PTH 1–34 100,000 pg/mL 3.9 0.00

PTH 39–68 100,000 pg/mL 4.8 0.00

PTH 39–84 100,000 pg/mL 14.8 0.01

PTH 44–68 100,000 pg/mL 4.5 0.00

PTH 53–84 100,000 pg/mL 4.8 0.00

PTH 7–84 1000 pg/mL 925.2 92.5%

 \* NA = Not Applicable. Concentration was below the measuring range of the test.

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