**Progesterone 4840 CH-184**

**Principle:**

The VITROS Progesterone test is performed using the VITROS Progesterone Reagent Pack and the VITROS Progesterone Calibrators on the VITROS 5600 Integrated System using Intellicheck® Technology. A competitive immunoassay technique is used. Progesterone present in the sample competes with a horseradish peroxidase (HRP)-labeled progesterone for a limited number of binding sites on a biotinylated rabbit anti-progesterone antibody presented in the liquid phase. The effects of binding proteins are eliminated by use of an appropriate blocking agent. The biotinylated rabbit anti-progesterone antibody is captured by streptavidin coated on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. 8 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 indirectly proportional to the concentration of progesterone present.







**Clinical Significance:**

The main sites of progesterone production are the adrenal cortex, ovaries, and corpus luteum following ovulation and the placenta by the twelfth week of pregnancy. 1 ‑ 3 Circulating progesterone is bound to several serum proteins including albumin and corticosteroid binding globulin. The physiologically active free hormone represents approximately 3% of the total progesterone concentration. 4 Measurement of serum progesterone is useful in the investigation of ovarian functionwhere disorders of ovulation are responsible for infertility in 15–20% of patients, and for predicting ovulation in inducedcycles, where concentrations are generally higher than normal. 5 Properly timed measurements of progesterone can be used in the diagnosis of patients with recurrent and threatened abortion in the first ten weeks of gestation. 6 , 7 Corpus luteum dysfunction is indicated by lower than normal progesterone concentrations.

**Specimen Collection and Storage:**

**Patient Preparation;**

 No special patient preparation is necessary.

**Specimens Recommended:**

 • Heparin plasma

**Specimens Not Recommended:.**

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

**Specimen Collection and Preparation**

 **•** Collect specimens using standard procedures. 12 , 13

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

 • The VITROS Progesterone test uses 25 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.

**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.

 • Plasma samples may be stored for up to 7 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, bacterial; binds ≥3ng biotin/well)

 • 10.0 mL conjugate reagent (HRP-progesterone, 70 ng/mL) in buffer with bovine serum albumin and antimicrobial agent

 • 10.0 mL biotinylated antibody reagent (biotin-rabbit polyclonal anti-progesterone, binds ≥70 pmol progesterone/mL) in buffer with bovine serum albumin, bovine gamma globulin 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 onto the system.

• Handle the reagent pack with care. Avoid the following:

 – allowing condensation to form on the pack

 – causing reagents to foam

 – agitation of the pack

Reagent Pack Storage and Preparation



 • The VITROS Progesterone 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 Progesterone Calibrators 1, 2 and 3 (freeze-dried progesterone in human serum with antimicrobial agent, reconstitution volume 1.0 mL); nominal values 0.0; 4.25 and 120 nmol/L (0.0; 1.33 and 37.7 ng/mL)

 • 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 (36–46 °F) as soon as possible after use, or load only sufficient for a single determination.

**Calibrator Storage and Preparation**

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VITROS Progesterone Calibrators are supplied freeze-dried.

 • VITROS Progesterone Calibrators are suitable for use until the expiration date on the carton when 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 Progesterone test uses 25 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:**

**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 Progesterone 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 Integrated Systems.

**QUALITY CONTROL:**

Refer to the Chemistry Quality Control Procedure for Specifics.

**Quality Control Material Selection**

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Progesterone 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. 14

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

**PROCEDURE:**

Materials Provided

 • VITROS Immunodiagnostic Products Progesterone Reagent Pack

 • VITROS Immunodiagnostic Products Progesterone Calibrators

Materials Required but Not Provided

 • VITROS Immunodiagnostic Products Signal Reagent

 • VITROS Immunodiagnostic Products Universal Wash Reagent

 • VITROS Immunodiagnostic Products High Sample Diluent A Reagent Pack

 • Quality control materials such as VITROS Immunodiagnostic Products RE Controls

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

 • Calibrated pipette, distilled water and sample containers for reconstitution of VITROS Progesterone Calibrators

**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**

 Serum or plasma (EDTA or heparin) samples with concentrations greater than the measuring range may be automatically diluted on the system up to 10-fold (1 part sample with 9 parts diluent)] by the VITROS 5600 with the VITROS High Sample Diluent A Reagent Pack prior to test. Refer to the VITROS High SampleDiluent A Reagent Pack instructions for use.

**Default Test Name**

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

**RESULTS:**

Results are automatically calculated by the VITROS 5600.

Reporting Units = ng/mL

**Reference Interval**

Ovulatory Cycle Range

 Follicular 0.14-2.03 ng/mL

 Periovulatory 0.40-4.47 ng/mL

 Mid Luteal 5.22-22.7 ng/mL

 Luteal 1.42-16.6 ng/mL

Pregnant Females Range

1st Trimester (4-13 weeks gestation) 6.57-40.3 ng/mL

2nd Trimester (13-24 weeks gestation) 9.66-62.3 ng/mL

3rd Trimester (25-36 weeks gestation) 24.5-334 ng/mL

 Post Menopausal Females 0.15-1.04 ng/mL

Normal Males 0.21-1.54 ng/mL

**PROCEDURAL NOTES:**

**Known Interferences**

The VITROS Progesterone test was evaluated for interference consistent with CLSI document EP7. 15 Commonly encountered substances were tested on 3 lots of reagents. The following compounds, when tested, caused the bias shown at the concentration indicated.

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

Interferent Interferent Concentration Conc.\* Bias\*\*

Dipyrone\* 1000 mg/mL 8.93 1.58

\* Average concentration of replicate determinations using 2 different lots of reagent.

\*\* Estimate of the average difference observed.

**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.

• Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. 16 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 progesterone concentrations in vivo. For additional information, refer to one of the published summaries. 17 ‑ 19

• Biotin levels in serum remain elevated for up to 24 hours after oral or intravenous biotin administration. 20

**Performance Characteristics**

**Limit of Detection**

The Limit of Detection (LoD) for VITROS Progesterone is 0.253 nmol/L (0.080 ng/mL), determined consistent with NCCLS document EP17 21 and with proportions of false positives (α) less than 5% and false negatives (β) less than 1%; based on 698 determinations, with 1 blank and 5 low-level samples. The Limit of Blank (LoB) is 0.085 nmol/L (0.027 ng/mL).

For the VITROS 5600 Integrated System, the LoB and LoD were verified consistent with NCCLS document EP17. 21 The data presented are a representation of the product performance.

Limit of Blank and Limit of Detection

LoB ng/mL\* LoD ng/mL\*\*

 0.027 0.080

\* 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 5% and 1% respectively; based on 698 determinations, with 1 blank and 5 low-level samples.

**Accuracy (Method Comparison)**

Accuracy was evaluated consistent with NCCLS document EP9. 22 The plots and table show the results of a method comparison study using patient samples from a variety of clinical categories analyzed on the VITROS ECi/ECiQ Immunodiagnostic System using VITROS Progesterone test lot numbers below 750 compared with those analyzed using the VITROS Progesterone test lot numbers 750 and above. The relationship between the 2 methods was determined by

Deming regression. 23

The table also shows the results of method comparison studies 24 using patient serum and plasma samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with those analyzed using the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System. The relationship between the 2 methods was determined by Deming regression. 23





**Precision**

VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System

Precision was evaluated consistent with NCCLS document EP5. 26 Two replicates each of 3 freeze-dried control samples 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 Progesterone test was evaluated for interference consistent with CLSI document EP7. 15 Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at progesterone concentrations of 5.03–28.0 nmol/L (1.58–8.80 ng/mL).



**Cross-Reactivity**

The cross-reactivity of the VITROS Progesterone test was evaluated by adding the following substances to control samples containing no progesterone.



 \* ND = Not Detectable. Concentration was below 0.25 nmol/L (0.08 ng/mL).

Cross-reactivity was expressed as the mean result obtained for the cross-reactant pool divided by the cross-reactant concentration in percentage term.

% Cross-reactivity = Mean Result for the Cross-reactant Pool

 Concentration of Cross-reactant × 100

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