2. Precision: Qualisa™ Progesterone was evaluated with licensed external Quality controls for Precision Studies & following is the data:

<table>
<thead>
<tr>
<th>Controls</th>
<th>No. of tests</th>
<th>Mean Control values with Qualisa™ Progesterone</th>
<th>Coefficient of Variable (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>0.783</td>
<td>6.49</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>12.16</td>
<td>5.98</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>27.10</td>
<td>4.05</td>
</tr>
</tbody>
</table>

B) External Evaluation: Qualisa™ Progesterone ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation Qualisa™ Progesterone ELISA has demonstrated 95% correlation with the reference method.

*Data file: Zephyr Biomedical (A Division of Tulip Diagnostics Pvt. Ltd.).

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY


SYMBOL KEYS

- Temperature Limitation
- Contact Instructions for use
- Date of Manufacture
- Lot Number
- Batch Number
- Material provided with the test kits:
  - Coated Microwells: Microwells coated with Goat Anti-Rabbit IgG.
  - Rabbit Anti-Progesterone Reagent. Ready to use.
  - Progesterone-HRP Conjugate Concentrate (11X).
  - Progesterone-HRP Conjugate Diluent.
  - TMB Substrate. Ready to use.
  - Stop Solution. Ready to use.
  - Progesterone Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer via label.
- Wash Buffer Concentrate (20X).

MATERIALS AND COMPONENTS

- Precision pipettes: 10µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. Qualisa™ Progesterone kit is stable at 2-8°C up to expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.

3. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.

**SPECIMEN COLLECTION**

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Serum only should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

**PRECAUTIONS**

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Do not mix components of one kit with another.
5. Do not use kit after the expiry date.
6. Do not use new tip for each specimen and reagent.
7. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
8. Use absorbent sheet to cover the working area.
9. Neutralize acid containing waste before adding hypochlorite.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.

**REAGENT PREPARATION**

1. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
3. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

**TEST PROCEDURE**

1. Secure the desired number of coated wells in the holder. Dispense 25 µl of standards and serums into the appropriate wells.
2. Dispense 50 µl of rabbit anti-progesterone reagent into each well, followed by 100 µl of Working Progesterone-HRP Conjugate reagent into each well. Incubate at room temperature (18-25°C) for 20 minutes. Thoroughly mix for 30 seconds.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense 100 µl of TMB substrate into each well. Incubate at room temperature (18-25°C), in the dark, for 20 minutes.
5. Stop the reaction by adding 100 µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.

**CALCULATION OF RESULTS**

Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of Progesterone in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

**Example of Standard curve**

Results of a typical standard run with optical density reading at 450nm (ref 600 – 700nm) shown in the Y axis against Progesterone concentrations shown in the X axis.

**Expected Ranges of values**

Each laboratory should establish its own normal range based on the patient population. The Progesterone EIA was performed on randomly selected outpatient clinical laboratory samples. The following information is cited from reference #9.

- **Males**: adult 0.13 – 0.97 ng/ml, Prepubertal (children) 0.70 – 0.52 ng/ml
- **Females**: follicular phase 0.15 – 0.70 ng/ml, luteal phase 2.00 – 25.0 ng/ml, post menopause 0.06 – 1.60 ng/ml
- **Pregnancy**: 1st trimester 10.3 – 44.0 ng/ml, 2nd trimester 19.5 – 82.5 ng/ml, 3rd trimester 65.0 – 229 ng/ml

The minimum detectable concentration of Progesterone by this assay is estimated to be 0.2 ng/ml.

**PERFORMANCE CHARACTERISTICS**

A) Internal Evaluation:

1. In an internal Study Qualisa™ Progesterone was evaluated against commercially available licensed kit with 90 random clinical samples and Qualisa™ Progesterone has demonstrated 95% clinical correlation with the commercially available licensed kit.