SCIMEDX CORPORATION

Technical Bulletin

.ELISA

A NEW CLASS OF ELISA TESTING

SCANLISA RBP ELISA

INTENDED USE

The SCIMEDX Corporation SCANLISA® Retinol Binding Protein (RBP) Assay is intended to quantify RBP in human serum as an aid to assess the Vitamin A status of a population in low-resource settings.

CLINICAL SIGNIFICANCE

Vitamin A deficiency (VAD) is a global health issue, especially in developing countries. While it is generally well known that vitamin A deficiency leads to blindness, vitamin A is also essential for normal functioning of the immune system. Vitamin A-deficient children have a significant incerease in mortality rate (25%) from a range of childhood ailments such as measles, malaria or diarrhoea.

Current methods to assess the extent of VAD among populations are expensive, time consuming and require the skills of highly trained technicians. Public health planners and researchers need simpler, less expensive methods to inform public policies and promote well-targeted vitamin A intervention programs.

RBP is a surrogate marker for retinol because of the close correspondence between retinol and RBP. A rapid and cost-effective quantitative enzyme-linked immunoabsorbent assay (ELISA) for detection of RBP has been developed. The assay takes approximately 40 minutes to complete and, using a reference panel of sera, test accuracy was found to be within 4% of expected values through the calibrated range of 0.48-1.92 micromol RBP/L (10-40 micro g RBP/mL). The RBP EIA provided linear results between 0.43 and 1.80 micro mol RBP/L (9 and 38 micro g RBP/mL). A recent study conducted in Thailand using this kit demonstrated a close correlation between RBP EIA levels and retinol levels as measured by HPLC.

The RBP-EIA is an antigen competition assay that can detect and quantify RBP from human serum, which exists in a 1:1 ratio with retinol, making RBP an ideal surrogate marker. The test uses purified human RBP adsorbed to the microtest strip wells to compete with natural RBP found in serum. To perform the assay, the specimens and control calibrator sera are added to individual wells. A monoclonal anti-RBP antibody, conjugated with the enzyme horseradish peroxidase (HRP), is then immediately added. The test is incubated at room temperature (18°C - 24°C) for 15 minutes and is then washed. Enzyme substrate is added, incubated at room temperature for 10 minutes, after which the reaction is stopped with acid. The test is immediately read on a plate reader, and the results are calculated based on values obtained from the calibration curve.

KEY FEATURES

- Simple and easy to use
- Rapid results in 40 minutes
- High throughput
- Accurate, reproducible and precise results
- Quantitative
- Less costly than traditional methods

BASIC ASSAY PROCEDURE

1. Dilute the calibrator concentrate to corresponding calibrator values in tubes, according to package insert.
2. Gently mix the calibrator dilutions just prior to use.
3. Dilute the Vitamin A positive (sufficient) and negative (deficient) controls 1:10 in assay diluent. Gently mix the dilutions.
4. Dilute patient samples 1:25 also in assay diluent. Gently mix the dilutions.
5. Dilute the antibody conjugate concentrate in assay diluent to the suggested conjugate strength for use in the assay, as stated in the Package Insert.
6. Using fresh pipette tips, transfer 100 ul of each calibrator, control and patient sample dilution from the dilution tubes into corresponding wells of the test plate(s).
7. Immediately add an equal volume (100 ul per well) of the diluted conjugate from step 4 to these wells. Cover the strip wells with plate sealer and incubate at room temperature (18 - 25 deg C) for 15 minutes, mixing gently at 10 minutes by slight tapping of the plate frame.
8. Carefully remove plate sealer and aspirate the wells. Wash the wells 5 successive times by adding and aspirating wash buffer to and from the wells. After the last wash, tap plate face down on paper towel or absorbent material to remove any remaining wash buffer in the wells.
9. Add 200 ul of TMB substrate to the wells, cover with plate sealer and allow to incubate at room temperature for 10 minutes.
10. Carefully remove sealer and add 100 ul of stop solution to each well. Immediately read absorbance or optical density (OD) of plate contents using an ELISA plate reader at 450 nm.

Available Tests

RBP96	96 tests

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