

Materials Required but not Provided

10 µl Micropipette
Timer and Test Tube Rack
DVM Rapid Test™ Instrument

Procedure (DVM Rapid Test™) (Note: A pictorial procedure is available in your DVM Rapid Test™ Operation Manual).

1. Bring the patient samples, control, reagent tubes and reagent blank tube to room temperature.
2. Label pre-loaded reagent tubes with sample I.D. number or control and remove cap (save cap).
3. Using a separate yellow pipette tip for each sample to be tested, add 10 µl of patient serum/plasma or control plasma into the corresponding pre-labeled reagent tubes. Recap tubes and gently mix by inversion (5 times).
4. Set a timer and incubate at room temperature for 10 minutes.
5. Turn on the DVM Rapid Test™ by pressing the "ON/OFF" button. Press "ON/OFF" a second time when "Model - 942, %T" appears in the LCD screen, to activate the autoblack function. The screen should read "AUTOBLANK ON".
6. During the ten minute incubation step and at the "INSERT BLANK" prompt, insert the Reagent Blank tube into the cuvette well. The display will read "READING BLANK" followed by "REMOVE BLANK". The instrument is now zeroed and ready to measure test samples. Remove and save the reagent blank tube.

NOTE: DO NOT DISCARD THE REAGENT BLANK TUBE UNTIL YOU HAVE USED ALL THE TESTS FOR AN INDIVIDUAL KIT.

7. After the ten minute incubation is completed, gently mix all samples by inversion (5 times), and then insert the first sample into the cuvette well (display reads "INSERT SAMPLE"). The unit will read the sample and displays a %T value on the LCD. Record this value on a copy of the Data Log sheet included your DVM Rapid Test™ Data Manual. Remove the sample tube and display will read "INSERT SAMPLE".
8. Repeat Step 7 above until all samples and/or controls have been read. Instrument will automatically turn off after being idle for more than 15 minutes.
9. Look up the concentration that corresponds to the %T value from the chart included with your DVM Rapid Test™ Data Manual.

Quality Control

The reliability of test results should be monitored whenever patient samples are assayed using a quality control material analyzed in the same manner employed for the unknowns. We suggest the use of the Camelid IgG Control provided with the kit.

Expected Values

Normal values for Camelid IgG vary greatly. The range for healthy adults is 1200 to >2000 mg/dl.

As an indicator of Failure of Passive Transfer (FPT) in crias:

Greater than or equal to 800 mg/dl = Adequate IgG levels
400 - 800 mg/dl = Reduced immunity and possible increased risk of infections

Less than 400 mg/dl = Positive FPT, greatly reduced immunity and increased risk of severe health complications

Performance

Linearity: Camelid IgG assay is linear from 0 to 3000 mg/dl.

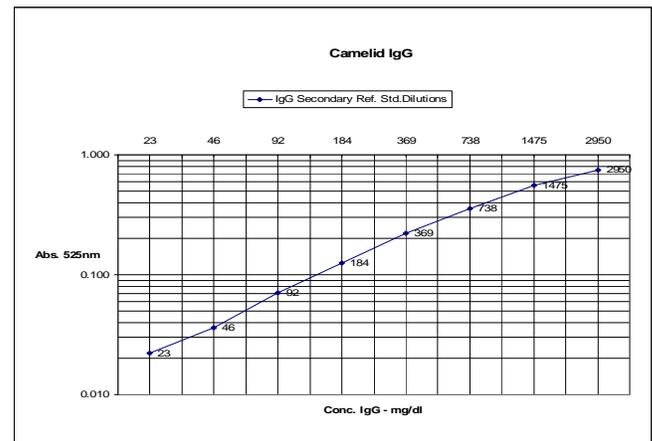


Figure 1 - Standard Curve for Camelid IgG TIA

References

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3. Jorgensen D. Llama Immunoglobulin G (IgG), and its role as an indicator of health status. By Published in "Llama Life," April 1991.
4. White DG and Andrews AH. (1986) Adequate concentration of circulating Colostral proteins for market calves. *Vet Rec* 119:112 - 113.
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Camelid Serum/Plasma IgG

Turbidimetric Immunoassay

Intended Use

Quantitative determination of Camelid IgG in serum or plasma by Turbidimetric Immunoassay.

Summary and Explanation of Test

In mammals, IgG is the major class of proteins called antibodies, the function of which is to provide immunity from foreign material. The measurement of plasma IgG levels in newborn ruminants (including camelids, horses and cows) around 24 hours after birth is an indication of passive transfer of immunity from the mother to the newborn. These neonates are born with little or no circulating IgG. Neonatal immunity to infectious agents requires the uptake and absorption of maternal antibodies from colostrum (mother's first milk) and is known as passive transfer of immunity. Partial or complete failure of passive transfer (FPT) occurs in up to 10% of all newborn ruminants, and these animals are at a high risk of illness or death.^{1, 2, 3} Several studies have identified plasma levels of IgG as indicators of successful passive transfer. Greater than 800 mg IgG per 100 ml of plasma (mg/dl) is considered an adequate level of immunity. Between 400 mg/dl and 800 mg/dl may be adequate, but the crias may be at risk depending on where the IgG levels are within this range. IgG levels below 400 mg/dl are indicative of FPT. Rapid identification of low plasma IgG levels and the severity of the IgG deficit is critical both in determining the need for IgG supplementation and the level of treatment necessary. Further, post treatment testing allows a timely evaluation of the success of IgG supplementation.

Passive transfer has been assessed by methods such as refractometry, total protein measurements and sodium sulfite turbidity testing; however, these methods have been documented to be highly variable and lacking specificity.^{4, 5, 6} The radial immunodiffusion test (RID) method developed by Mancini⁷ and others⁸ is commonly used to measure serum/plasma IgG concentration, although it requires a long diffusion time (24 to 72 hours) and is labor intensive. The manual turbidimetric immunoassay (TIA) described herein and a modification of a previously reported automated assay⁹ reduces analysis time to ten minutes and can be performed on-site using an inexpensive fixed wavelength portable spectrophotometer.

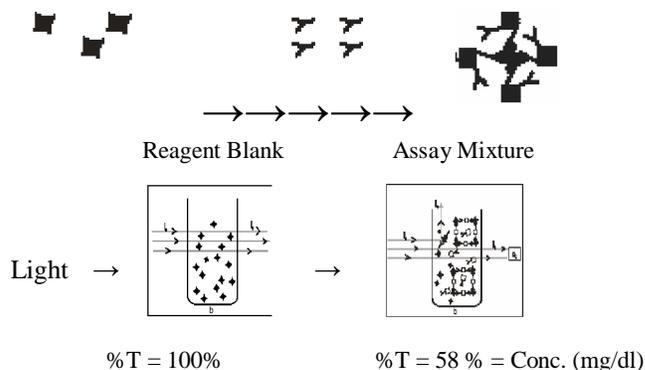
Principle

Measurement of immune complexes by Spectrophotometry (a.k.a. Turbidimetry) is unlike classical biochemistry where the reactants are clear and endpoints are expressed as absorbances as the behavior of light differs for solutions containing suspensions or particulates.^{10, 11} Such insoluble immune complexes are formed as a reaction between antigens (Camelid IgG in the serum/plasma) and antibodies (Goat Anti-Camelid IgG) and takes place in a polyethylene glycol based medium. Light of suitable wavelength is allowed to pass through the reaction solution containing only the antibodies (Reagent Blank) and the initial absorbance of light is measured and set as a zero point (Blanking). Subsequently the test sample containing the antigen (analyte) is then added to the antibody reagent solution mixed and allowed to react. An agglutination reaction takes place when a single molecule of antibody binds to at least two corresponding binding sites on

different antigen particles. As the reaction proceeds, the agglutinating particles aggregate and form immune complexes. Immune complexes increase in size, become larger, resulting in an increase in turbidity and the decrease in the quantity of the incident light that is transmitted (%T) as the reaction proceeds. Spectrophotometers read this decrease in the intensity of the transmitted light (%T). The decrease in %T is related to the concentration of antigen (IgG) in a linear relationship over a specific range of analyte concentration (Assay range).

Detection Principles in Turbidimetry

Anti-IgG Reagent + Analyte (Plasma IgG) = Immune complexes



Reagent Storage and Stability:

All reagents are stable at 2 - 8°C for 24 months from date of manufacture. All reagents are stable until the expiration date stated on the labels. Do not use the reagents past their expiration date. Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability. Bring reagent tubes and control to room temperature before use.

Warnings and Precautions

All reagents contain 0.1% Sodium Azide as a preservative. Sodium Azide may react with copper or lead plumbing to form explosive metal azide build up. On disposal, flush with large volumes of water.

Specimen Collection and Preparation

Serum or plasma collected with EDTA or heparin can be used in the assay. Serum or plasma should be separated from the cellular components of whole blood as soon as possible. Separated serum or plasma should be stored refrigerated if it is not to be assayed immediately. Grossly hemolyzed samples should not be used for this procedure.

Interfering Substances

The following substances have no significant effect on the accuracy of this procedure at the concentrations stated.

- Hemoglobin ≤ 100 mg/dl
- Triglycerides ≤ 500 mg/dl

Materials Provided

- 10 or 20 - Preloaded Single Test IgG Reagent Tubes
- 1 - Preloaded IgG Reagent Blank Tube
- 1 - Vial of Camelid Control Serum