Coombs' Testing and Its Diagnostic Significance in Dogs and Cats

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KEYWORDS
• Blood • IMHA • Coombs' test • Antiglobulin test
• Hematology

The Coombs' test, also known as the antiglobulin test, is used to detect antibody and complement on the surface of red blood cells (RBCs). The test was developed by the veterinarian Robin R.A. Coombs in 1945, and was originally used to detect antibodies against antigens in the human Rh blood group system.[1,2] It is used most often in veterinary medicine to aid in the diagnosis of immune-mediated hemolytic anemia (IMHA). An extensive review of the Coombs' test in veterinary medicine has been previously published.[3] The current article provides a brief overview and in addition focuses on more recent studies, particularly those examining test performance, diagnostic significance, and alternate technologies.

TEST METHODOLOGY

The antiglobulin reagents used in the Coombs' test are species-specific and are generally produced in rabbits or goats. Polyvalent reagents contain a combination of anti-IgG, anti-IgM and anti-C3 and can detect immunoglobulin and complement on the surface of RBCs. Monovalent reagents are directed against individual immunoglobulins (Ig) or complement (generally C3 or C3b). The reagents are typically adsorbed with normal RBCs from the target species to remove any heterologous antibodies that may be present. The activity or agglutinating potential of polyvalent and monovalent reagents can vary, dependent on their mode of preparation.

Two forms of the antiglobulin test can be used in veterinary medicine. The direct antiglobulin test (DAT) detects Ig and/or complement that is bound to patient RBCs, and is frequently used in the diagnosis of IMHA (**Fig. 1**). The indirect antiglobulin test (IAT) detects the presence of unbound antibody in the serum and is infrequently used in veterinary medicine, being reserved for commercial blood typing and occasionally

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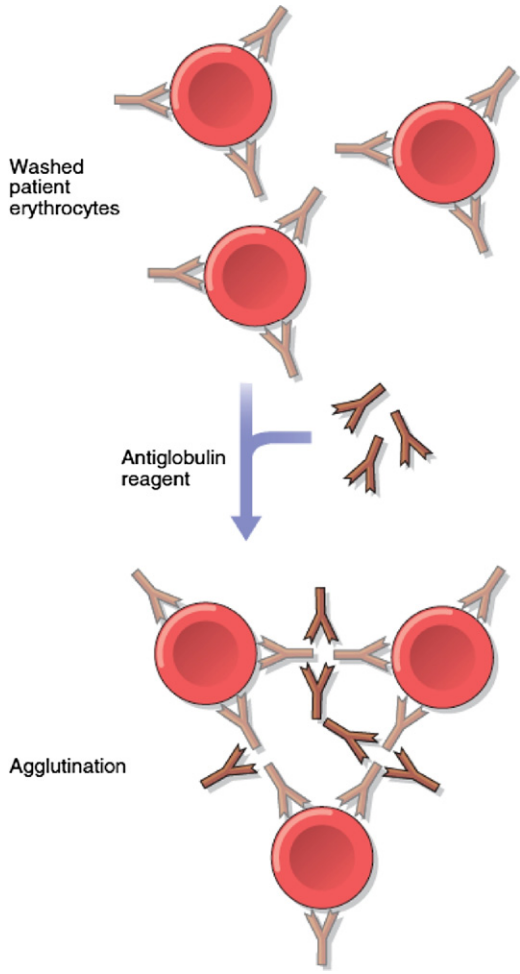

Fig. 1. Principle of the Coombs' test. Antiglobulin reagent reacts with immunoglobulin or complement bound to RBCs, forming a lattice that appears as agglutination. (*From* Day MJ. Immune-mediated anemias in the dog. In: Weiss DJ, Wardrop KJ, editors. Schalm's veterinary hematology. 6th edition. Ames (IA): Wiley-Blackwell; 2010. p. 216–25; with permission.)

used for crossmatching. Standard procedures for the DAT and IAT using tubes are described in **Tables 1** and **2**. The test can also be performed in microtiter plates, which use smaller volumes of reagent and RBCs per well and allow for a greater number of tests and dilutions of antiglobulin reagent to be performed. Typically, one or more wells of the microtiter plates contain the negative reagent control and the remaining wells contain a washed suspension of RBCs and antiglobulin reagent at increasing dilutions. Following incubation, wells are considered negative if they contain a button of RBCs that stream when the plate is tilted. Positive wells have a matte formation that does not stream. The term "full Coombs test" has been used to describe a Coombs' test performed in microtiter plates, fully titrated with both polyvalent and monovalent reagents and tested at both 4°C and 37°C.[4,5]