Table I. Titre of anti-chicken antibodies (serum)

Dilution	No. of twin shot rabbits				No. of multiple shot rabbits			
	1	2	3	4	1	2	3	4
1:2	+	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+	+
1:8	+	+	+	+	+	+	+	+
1:16	+	+	+	-	+	+	+	+
1:32	-	-	-	-	+	+	-	+
1:64	-	-	-	-	-	-	-	-

contents, it was found that multiple shot regime was better for the production of anti-species antibodies than twin shot regime.

Before the determination of anti-chicken antibodies titres with AGPT, the conditions for the said test were optimized. Best results were obtained with 1.2% agar solution in veronal buffer placing for 16 h at refrigeration temperature. The well size and the distance between the central and peripheral wells were also optimized. The best results were obtained with 6 mm size of the well and with distance of 5 and 6 mm between central and peripheral wells. Though AGPT is generally used for qualitative indications of antibodies, but it was made fit for quantitative purpose using the serum dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128. These dilutions were prepared in microtitration plates by taking 50 µL of normal saline. The titres of twin shot rabbits were ranged from 1:2 to 1:16, while those of multiple shot rabbits ranged from 1:2 to 1:32 (Table I). The geometric mean titre (GMT) of multiple shot rabbits was 28 and that of twin shot rabbits was 14.

The effect of temperature (Table II) was observed on the rabbit anti-chicken antibodies to examine their stability against different temperature (-4, 18, 37, 45 and 60°C) and time combinations (2, 4 and 6 h). To see the effect, the structural stability was confirmed by applying AGPT. The results were positive for –4, 18, 37, 45 and 60°C recorded after 2 and 4 h. It was concluded that thermal treatment at any of these temperatures did not affect the stability of antibodies but after 6 h the results were negative for 60°C. It might be due to denaturing of antibodies.

Table II. Effect of temperature on anti-chicken antibodies (multiple shot serum)

Time (h)	Temperature (°C)				
	-4	-18	-37	-45	-60
2	+	+	+	+	+
4	+	+	+	+	+
6	+	+	+	+	+

Buffers of different pH values 3.0, 5.0, 5.2, 7.0 and 7.2 were used to see the pH effect on these antibodies. The result revealed that there was no effect of all studied pH belonging to phosphate buffer and carbonate buffer whereas only exception was with 5.2 pH value of carbonate buffer which was found effective on the activity of antibodies.

The pH value 3.0 and 3.5 of citrate and acetate buffer affected the antigen and antibody reactions. These results were in contradiction to the findings of Shimizu (1993) who studied the molecular stability of rabbits Igs and found that rabbits Igs were resistant to acid and other denaturant treatments.

Anti-species antibodies are the antibodies, which are produced by injecting antibodies of any one species in any other species. These antibodies are used in various immunodiagnostic tests including Indirect Flourscent Antibody Technique, Radioimmuno assay (RIA), and enzyme linked immunosorbent assay (ELISA) for the detection of antigen and antibodies (Weir, 1983).

It was observed that the anti-chicken anti-bodies were stable over a wide range of temperature (-4 to 60°C) and pH (5-9). So, it was concluded that these anti-bodies could be used in various immunodiagnostic tests including enzyme linked immunosorbent assay.

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