A Rapid Immunoblot Technique for Anti-Salmonella IgY Antibodies in Chicken Eggs

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Contamination of poultry with Salmonella spp. continues to be a major health concern. Enzyme linked immuno-sorbant assays (ELISA) are often used to evaluate eggs for the presence of related IgY anti-Salmonella antibodies. To avoid the long ELISA procedure, involving parts of two or three lab periods, we developed an accelerated method completed in one three-hour period. Initially, several serovar-specific lipopolysaccharide (LPS) antigens were bound to nylon membranes. After blocking, these were cut into strips, exposed to egg yolk solution taken by needle biopsy from single eggs, and developed with secondary antibody and chromogenic substrate. Results were observed the same day. In a 3-hour lab format, eggs were taken from many sources (chain stores, farmer's markets, farms, and organic or free-range growers). Each student used four eggs from each of three sources. Typical thesis statements, based on prelab discussion, called for comparison of IgY levels in eggs from two or more sources. And, since LPS antigens from many Salmonella enterica serovars with known O-antigen formulas were compared on each strip, hypothetical predictions were made regarding antigen cross-reactivity. After the initial lab, results were discussed and a lab report submitted. Over 90% of all samples tested, regardless of origin, were positive for some form of anti-Salmonella antibodies. Virtually all samples positive for Typhimurium LPS were positive for Enteritidis LPS, based on their similar O-antigen formulas [1, 4, 12] and [1, 9, 12], respectively; these showed no relationship to the Minnesota serovar with antigen formula [21]. During the follow-up week, student initiated technical improvements were tested and integrated into a revised protocol, providing each student with experience in design and improvement of the final protocol.

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