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| **Immune defense measured** | **Component** | **Assay name** | **Immunological background** | **Ref (s)** |
| Constitutive Innate  *BASELINE* | NAb – Complement activity | Hemolysis-Hemagglutination | Part of the innate immune system, but can be recruited by the adaptive system. C5b initates the membrane attack pathway which causes lysis of the target cell. Macrophages then clear the complement coated cells. Haemagglutination – tests for natural antibody (NAb) activity. Agglutination will occur if the antibody binds to a molecule present on the surface of RBCs. | Matson et al. 2005 |
| Complement activity | Complement-mediated lysis | Complement is an innate function, but also a strong bridge between innate and adaptive systems. Assesses the ability of complement to lyse foreign blood cells. | O’Neal and Ketterson 2012 |
| White blood cell counts (differentials) | Blood smears | Need only a very small blood sample. Can test for differences in total WBC, individual WBCs or H:L ratio. H:L ratio is less sensitive to short-term stress. | Owen et al. 2011 |
| Plasma lysozyme | Optical density measurements of the antimicrobial activity of plasma lysozyme | Lysozymes are enzymes that damage bacterial cell walls and cause hydrolysis. Lysozymes are part of the innate immune system and reduced levels have been associated with some diseases. | Millet et al. 2007 |
| Bacterial killing ability | Bacterial killing assay (BKA) | Considered a ‘course assay’ of innate immunity. Assesses the ability of immune factors in the blood to kill bacteria or yeast (usually Ecoli). Can be difficult to optimize. More plaque formations=less killing. Can use whole blood or plasma (just changes the Ecoli form you use). | Millet et al. 2006; Ardia 2008 |
| Induced Innate | Acute phase response Fever | Cloacal temperature following injection with lipopolysaccharide (LPS) | Indicates a fever response to the LPS injection. Fever could lead to the production of reactive oxygen species, ultimately causing immune-pathological tissue damage. LPS is lipopolysaccharide is an endotoxin (found in gram negative bacterias) and elicit strong immune responses. | Coon et al. 2011 |
| Acute phase protein-Haptoglobin | Plasma haptoglobin or mannan-binding protein following injection with LPS | Acute phase proteins are synthesized in the liver and generally increase after infection as part of the systemic inflammatory response. The acute-phase proteins play a role in the early innate immune response. | Millet et al. 2007 |
| Constitutive Adaptive  *BASELINE* | Total IgG | IgG Elisa | Nonspecific antibody response which is indicative of a specific antibody response. |  |
| Induced Adaptive | Antibody production Humoral | Immunization - antibodies IgM and IgG measured by ELISA after 1o and 2o exposure to antigen keyhole limpet haemocyanin (KLH) | A protein found in the hemolymph of limpets; therefore a novel noninfectious antigen to avian species. First and second injections measure the primary and secondary antibody response, respectively. | Ebel et al. 2002; Fassbinder-Orth et al. 2009 |
| Other… | Toll-like receptor | Measure before and after injection with LPS | Mediator(s) of inflammatory response. TLRs are receptors for ‘conserved microbial elements’. Occur primarily on macrophages, dendritic cells and heterophils. Specifically, TLR-2 and 4 are ‘strong instigators of APRs and recognize components of bacteria’. | Martin et al. 2014. |
| Cytokines (IL-6) | Measure before and after injection with LPS | A pro-inflammatory cytokine. It is secreted by T cells and macrophages. IL-6 also plays in mediating fevor and the acute phase response. | Martin et al. 2014. |

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| **Immune defense measured** | **Component** | **Assay name** | **Amount of blood/plasma needed** | **Technique/Materials** | **Protocol available** |
| Constitutive Innate  *BASELINE* | NAb – Complement activity | Hemolysis-Hemagglutination | 20-50ul plasma | Amount of plasma depends on the protocol used, but would need to be consistent throughout the study. | Yes, but need to optimize |
| Complement activity | Complement-mediated lysis | 10-20ul plasma | Would want to run as many samples as possible within 2week time span as Rabbit RBC’s expire in 2 weeks and are expensive. | Yes, but need to optimize |
| White blood cell counts (differentials) | Blood smears | Maybe 5ul whole blood | Tap a dot of blood onto the microscope slide prior to spinning down. Stain with Giemsa. | Yes |
| Plasma lysozyme | Optical density measurements of the antimicrobial activity of plasma lysozyme | 10ul in duplicate=20ul plasma | ‘Read out’ clarity (killing of the lysozyme) vs. opacity (unkilled bacteria) is somewhat subjective | Yes |
| Bacterial killing ability | Bacterial killing assay (BKA) | 10-15ul whole blood or plasma | Would take some time to optimize, but not impossible. Would also need to determine which ‘type’ we want to use as some BKAs are simpler than others. | Yes, but need to optimize |
| Induced Innate | Acute phase response Fever | Cloacal temperature following injection with lipopolysaccharide (LPS) | NA |  | Yes |
| Acute phase protein-Haptoglobin | Plasma haptoglobin or mannan-binding protein following injection with LPS | 7.5ul (should run in duplicate/triplicate) |  | Yes |
| Constitutive Adaptive  *BASELINE* | Total IgG | IgG Elisa | 5-10ul plasma | Prepare plate overnight and then a few hour-long incubations. | Yes |
| Induced Adaptive | Antibody production Humoral | Immunization - antibodies IgM and IgG measured by ELISA after 1o and 2o exposure to antigen keyhole limpet haemocyanin (KLH) | Minimal (2-4ul) |  | Yes |
| Other… | Toll-like receptor | PCR | Minimal (2-6ul) | Measure before and after injection with LPS |  |
| Cytokines (IL-6) | PCR | Minimal (2-6ul) | Measure before and after injection with LPS |  |