GYROS

Application Report 205

Quantification of antigen-specific antibodies in serum

Detect specific antibodies in serum

- **Flexible assay format**
- **One sample per microstructure eliminates risk for cross-talk**

Detect antibody response at an early stage

- **Specific antibodies detected in nanoliter volumes of mouse serum**

Introduction

The overall objective of vaccination is to create a functional immune response that can protect the individual from primarily infectious diseases. The generation of an appropriate immune response depends on the composition and formulation of the vaccine. Characterization of the immune response in terms of quantity, affinity and immunoglobulin class is one way to evaluate vaccine efficacy.

Several assay principles can be used for quantification and characterization of specific antibodies in serum: competition assay, indirect antibody assay and antigen bridging antibodyassay (BA).

Together with the National Veterinary Institute (SVA), Sweden, a BA has been developed to monitor the progress of antibody responses in mice immunized with porcine parvovirus (PPV) vaccine preparations using Gyrolab.

A BA has the potential to give very precise results: only functionally active antibodies with defined antigen specificity are detected without interference from other antibodies present in serum. In addition, BA is excellent for monitoring total antibody responses since the assay is independent of immunoglobulin class and origin of antibodies, simplifying comparisons between species during vaccine development.

A BA performed using Gyrolab requires that the antigen can be labeled with a biotin-labeled capturing agent and fluorophore-labeled a detection reagent. In this study, biotin is used as capture agent and Alexa[1] is used as detection agent.

Thanks to miniaturization and the broad, four-log dynamic range, Gyrolab requires only nanoliters of critical sample and labeled reagents, up to a hundred-fold reduction compared to conventional formats. Automation and the unique flow-through design reduce `hands-on' time and speed up throughput, by providing identical running conditions for each reaction, generating up to 112 data points in less than 60 minutes.

Results

Flexible assay format

Gyrolab is an open platform that may be adapted to several assay formats. In a conventional antibody assay, a significant excess of the antigen is presented on the solid phase. However, to minimize the risk that bound antibody escapes detection in BA, which may happen when both binding sites of the antibody bind to immobilized antigen, the density of antigen on the solid phase must be lowered and optimized. A simple way of lowering the antigen density on the capture column is to mix biotinylated PPV antigen with a biotinylated protein not involved in immune reactions at dilutions giving concentrations that saturate the column capacity.

The first step in the assay development was to test the response values for different concentration ratios between biotinylated virus antigen and biotinylated BSA (see Table 1). A final combination of a 1/80 dilution of biotinylated PPV mixed with an equal volume of a 1/50 dilution of biotinylated BSA was selected to achieve a suitable PPV-binding capacity.

In the next step, the signal to noise ratio was optimized to give the highest possible response while keeping the consumption of valuable reagents to a minimum. The concentration of Alexa-labeled antigen preparation was titrated and the optimal concentration was calculated at 9 ng antigen per reaction.

	B*BSA						
B*PPV	1	1/4	1/8	1/16	1/32	1/64	1/128
1/10	33	53		61		61	
1/40	12	32		56		64	
1/80				49		73	
1/160	4.1	14	17	35	40	49	50
1/320				15		27	
1/640	1.2	4.5	4.3	11	11	22	16
1/1280			0.5	3.7	1.2	7.0	1.5

Table 1 Response values for different combinations of biotinylated PPV antigen and biotinylated BSA using constant amounts of rabbit anti-PPV and a constant dilution of Alexa-labeled PPV antigen. The darkest shaded value represents the combination with the highest response value.

Assay compatible with analyzing undluted serum samples

The standard curve shown in Figure 1 covers a dilution range of 1/5 to 1/78125 dilutions of a serum pool. The inter-assay variation (CV%) was < 7 % over a concentration range of more than 4 orders of magnitude based on repeated analyses of the standard curve on four separate occasions.

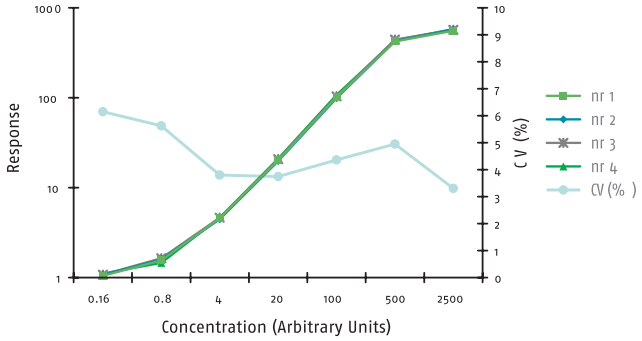

Figure 1 Sera from five mice were pooled and serially diluted to serve as a standard curve in the assay. The run was repeated on four consecutive days on the same Gyrolab instrument to illustrate the day-to-day variation. A concentration of 100 on the x-axis corresponds to a serum dilution factor of 125. On the left hand y-axis the fluorescence response is illustrated and on the right hand y-axis the CV (%) of 12 replicates is illustrated.

The BA was compatible with the analysis of samples even at dilutions of only 1/2, indicating the ability to detect very low levels of PPV-specific antibodies (Ref. 1).

The intra-assay precision was evaluated by aspirating 12 replicates of the standard pool of mouse a-PPV antibody diluted to 100 arbitrary units (125 fold dilution factor) and analyzing for anti-PPV antibodies (Figure 2).

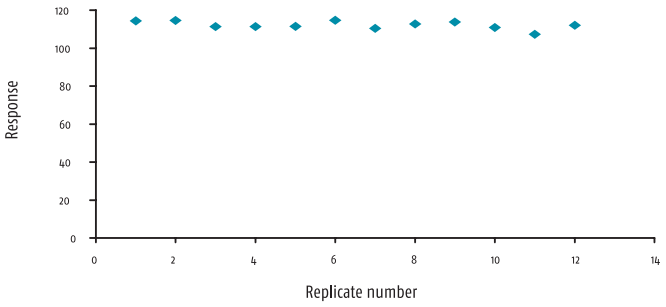

Figure 2 Intra-assay precision. Replicate determinations were performed for the standard pool of mouse a-PPV antibody diluted 125 times by aspirating 12 replicates of sample in one capillary of the instrument and further analyzed in neighboring Bioaffy CD microstructures for the amount of anti-PPV antibody. CV was 1.89 %.

Quantification of PPV antibodies in serum

A total of 234 samples were analyzed at a 1/25 dilution. Some samples were rerun at higher dilutions when indications of immunochemical saturation of the streptavidin column were evident. The results are illustrated in Figure 3, which displays the development of antibody concentration over the sampling period for each treatment group.

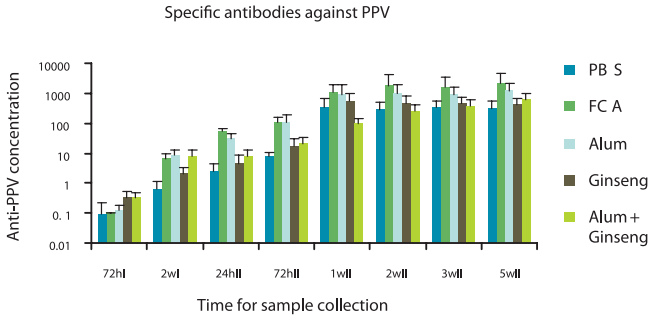

Figure 3 Quantification of anti-PPV in mouse serum samples. Groups of five mice were immunized with PPV together with five different adjuvants and evaluated for quantity of PPV specific antibodies in serum (Ref. 2).

Low reagent consumption

To minimize the hands-on time required for antigen-labeling, low reagent consumption is an advantage. For each reaction in the Bioaffy CD, only 305 nl is consumed. Table 2 illustrates the number of reactions that can be obtained from typical batches of biotinylated or Alexa-labeled antigen preparations.

Labeling procedure	Start amount (µg)	Final volume (µl)	Dilution factor	No of reactions
Biotinylation	50	60	1/160	31,500
Alexa labeling	90	90	1/40	11,800

Table 2 Reagent consumption expressed as the number of reactions that can be performed at the optimized reagent concentrations.

A total of 190 samples were analyzed for contents of PPV specific antibodies using an Indirect Antibody Assay (IAA) and BA. The results were compared and displayed in Figure 4. The correlation coefficient between the two methods was found to be r = 0.94.

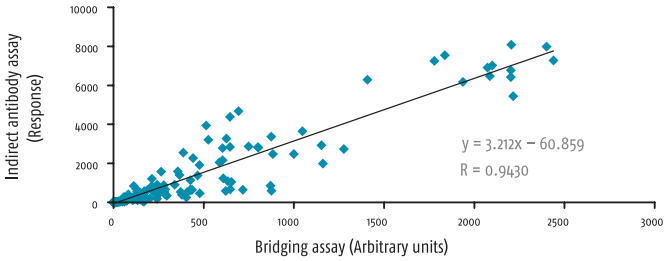

Figure 4 Correlation plot for 190 mouse serum samples determined for contents of PPV specific antibodies using IAA and BA (Ref. 3).

Conclusions

The successful collaboration with the National Veterinary Institute, Sweden, has demonstrated that Gyrolab is a suitable platform for quantification of antigen-specific antibodies in serum samples, an application requiring high specificity in combination with a low threshold. Gyrolab also offers the following benefits:

- **Broad dynamic range, permits detection of low amounts of antibodies obtained in early response during vaccine development**
- **Reduced consumption of valuable samples. The volume of blood taken from the mice can be reduced so that the animals recover quicker and can be bled more frequently. The precision of kinetic data is improved, and the fate of biomarkers and the effect of drugs can be monitored in more detail**
- **Open system that easily can be adapted to different assay formats such as BA, indirect antibody assay and sandwich immunoassay**

References

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2. Rivera E., Ekholm Pettersson F., Inganäs M., Paulie S. and Grönvik K.-O. "The Rb1 fraction of ginseng elicits a balanced Th1 and Th2 immune response." Vaccine 23, 5411–5419, 2005.

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