High Throughput use of the Haemagglutination Inhibition Assay During Phase 3 Clinical Trials Using Large Virus Panels

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Abstract

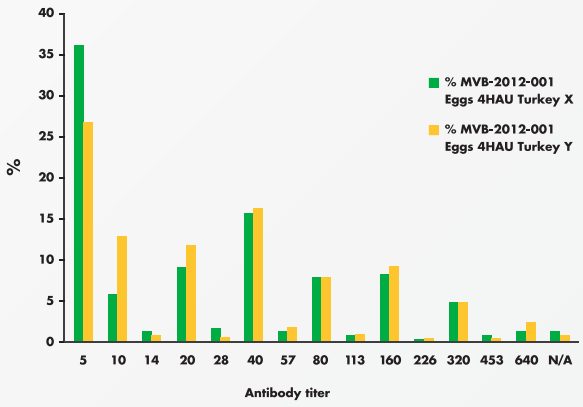
hVIVO has for many years carried out the Haemagglutination Assay (HAI) at a high rate of throughput for clients. This assay, based on the World Health Organisation standardised assay is fully validated to FDA and ICH guidelines and many procedures have been introduced to improve the quality and reduce the variability of this assay. This includes several inter-laboratory comparative studies. The poster displayed here highlights some of these procedural changes and results of some of these interlab comparisons, along with data on the performance of the HAI assay at hVIVO and some examples of where the assay has been used in a high throughput fashion.

HAI Assay: Reducing variation

The HAI assay is known to be poorly reproducible between laboratories and the results can also vary and be poorly reproducible based on the reagents used. To minimise the variation in the hVIVO HAI assay a number of improvements have been made. At hVIVO the HAI assay is performed using Turkey red blood cells (TRBC's). Tests performed previously at hVIVO demonstrated variation in HAI results can be seen when using blood sourced from different Turkeys (figure 1) and therefore we have introduced the qualification of the Turkeys that are used as the source of the red blood cells. Before any turkey is used for the HAI assay it is evaluated to ensure consistency in results. In addition all samples are tested in duplicate using red blood cells from two qualified Turkeys and the results averaged.

hVIVO have also Introduced rigorous operator qualification parameters and periodical reassessments to ensure that each operator is performing the assay in the same way. We have also introduced 3 human serum controls are used in assay to detect any issues with sensitivity towards human serum in addition to the recommended standard antisera controls.

Figure 1: Results demonstrating the variation in HAI titres observed using blood sourced from two different Turkeys using the same serum samples against A/Perth/16/2009



Inter-laboratory comparative studies

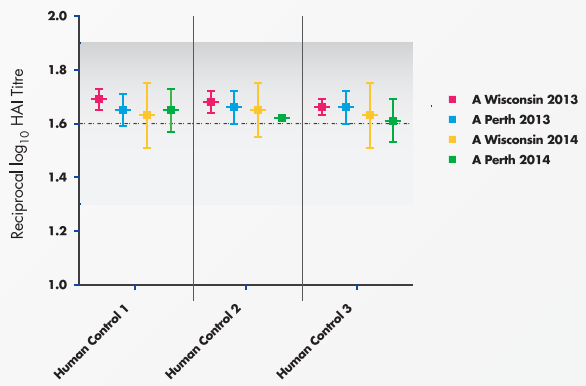
hVIVO have performed multiple inter laboratory comparative studies. This included a comparative study with a major Pharmaceutical company, where in total three comparative studies were performed using greater than 500 samples tested against up to 5 different flu strains. hVIVO had to pass the same stringent acceptance criteria used to qualify all this big pharmaceutical companies sites globally. Of all the companies that performed the evaluation hVIVO was the only one to pass these parameters.

Another study, conducted with a major UK University using 198 samples demonstrated a 94% agreement between the hVIVO and University HAI assay. The HAI assay is known to be poorly reproducible between laboratories, however the results from the two laboratory comparisons are more consistent than reported elsewhere (Wood et al, 1994, Stephenson et al, 2007).

HAI assay performance

As mentioned above hVIVO run additional antiserum controls in the HAI assay, in addition to standard antiserum controls The human serum control titres are monitored continuously (as are the animal antisera titres) and these results can be seen in figure 2

Figure 2: Performance of the hVIVO HAI assay against the 3 human control sera over the previous 2 years. Reciprocal log10 HAI titres for the three human serum controls are shown, with the expected titre represented by a line and the acceptable titre range shaded. As can be seen the confidence intervals are very small, demonstrating the repeatability of the assay



The expected titre of 1.6 log10 +/- 0.3 log10 reciprocal HAI titres (An arithmetic titre of 40 +/- a 1 in 2 dilution step- the acceptable variation for the assay) has been consistently observed over the previous 2 years, with the confidence intervals demonstrating the high level of repeatability observed.

hVIVO's influenza virus panels

hVIVO maintains a large repository of different virus stocks, which can be used as panels for clients to use in the evaluation of products or in screening of individuals for serum antibodies. The panel currently consists of:

- 35 Influenza A viruses (H1N1, H3N2, Recombinant H5N1) from 1933 to present
- 17 Influenza B viruses (representatives of both lineages) from 1987 to present

In addition hVIVO maintain a large number of serological samples as part of our screening process.

High throughput screening using the HAI assay

hVIVO, as a standard part of the screening process for our clinical trials undertakes an average of approximately 500 human serum samples are run per month (figure 3). In addition to this, to meet our clients' needs we have run a very large number of assays over a very short time period. One such study is represented graphically in figure 4, where approximately 25,000 assays were performed over a 7 week period.

Figure 3: Monthly number of samples analysed in the HAI as part of hVIVO's panel screening process to identify of serosuitable individuals for challenge

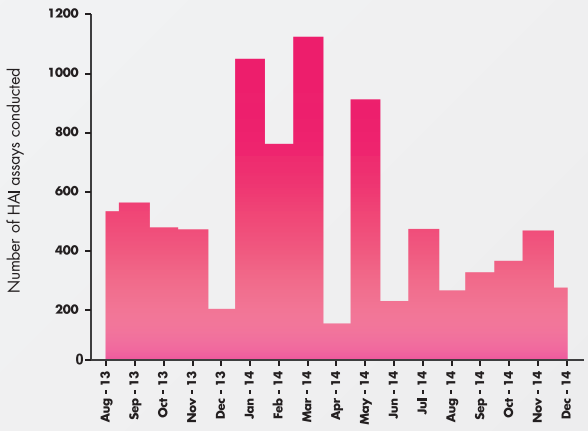


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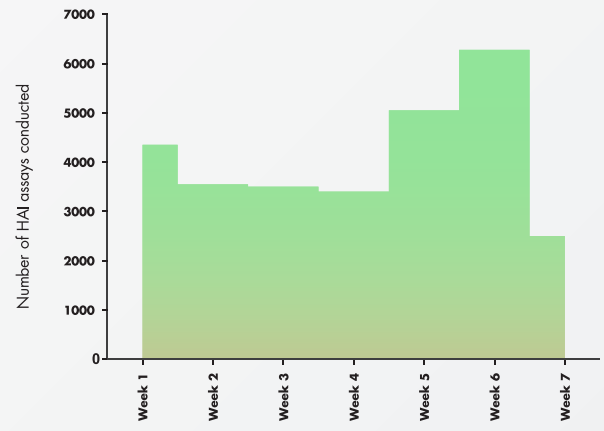
Figure 4: Number of HAI samples processed at hVIVO over a 7 week period for a client study



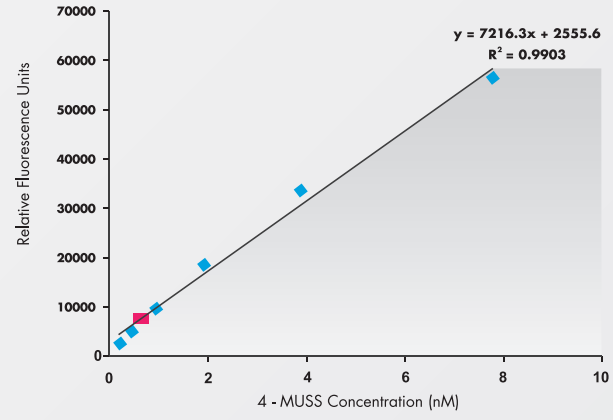
Conclusion

The data presented here shows the robustness and capacity of the hVIVO HAI assay, which has been optimised by the inclusion of various qualification methods for performance and constistency to ensure the very best results are delivered to our clients

And by the way...

hVIVO have recently optimised and validated a Neuraminidase Inhibition Assay (NAI) using the MU-NANA method. As for all hVIVO validations this was performed to FDA and ICH guidelines. The linearity of the assay as determined using a 4-MUSS standard is shown in figure 5.

Figure 5: Graph showing the linearity of the NAI assay using the 4-MUSS standard



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