SHORT COMMUNICATION

COMPARISON OF COMMERCIAL ELISA FOR DETECTION OF ANTIBODIES TO THE VIRAL CAPSID ANTIGEN (VCA) OF EPSTEIN-BARR VIRUS (EBV).

JODY L. MITCHELL, CATHERINE M. DOYLE, MEAGAN V. LAND, PETER L. DEVINE
PanBio Pty Ltd, Windsor, Queensland, Australia.

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Epstein-Barr Virus (EBV) is the causative agent of infectious mononucleosis (IM). The virus is primarily transmitted via saliva, with seroprevalence of approximately 90% in adults (Rocchi *et al.*, 1977; Yao *et al.*, 1985). Primary EBV infection in infants and young children is usually asymptomatic or at least very mild. In adults, IM is a self-limiting disease characterised by fever, pharyngitis, generalised lymphadenopathy and splenomegaly (Evans, 1976).

Traditionally, the serodiagnosis of EBV infection is based on the detection of heterophile antibodies. However, at least 10% of people with acute IM fail to produce heterophile antibody with this figure being much higher in children (Evans *et al.*, 1975; Sumaya and Ench, 1985). A more specific method of serodiagnosis of IM is the detection of EBV-specific antibodies that appear during primary infection. Most serodiagnostic tests are based on the detection of antibodies to the Viral Capsid Antigen (VCA) (Weidbrauk *et al.*, 1993; Field *et al.*, 1995). VCA antibodies of the IgG and IgM class become detectable early in the acute phase of infection. Anti-VCA IgM levels peak about 3 weeks after the onset of clinical disease, then rapidly decline to become undetectable. In contrast, VCA IgG antibodies persist for life (Youmans *et al.*, 1985; Nikoskelainen *et al.*, 1975).

Although the indirect immunofluorescence assay (IFA) is the standard method for detection of anti-VCA antibodies, it is not without limitations. IFA is difficult to standardize, time-consuming and not convenient for large scale screening (Wiedbrauk *et al.*, 1993). The development of enzyme-linked immunosorbent assays (ELISAs) for specific EBV antibodies has provided a sensitive and specific method that is objective and convenient for large scale screening (Hopkins *et al.*, 1982; Luka *et al.*, 1984) and assays for VCA are now available commercially.

Correspondence to: Dr Peter Devine, PanBio Pty Ltd, PO Box 7269, East Brisbane 4169, Queensland, Australia. Phone: 61-7-33571177, fax: 61-7-33571222.

In this study we have compared commercially available ELISAs for the detection of EBV VCA IgM and EBV VCA IgG (PanBio, Australia; Gull Laboratories, U.S.A.; Incstar, U.S.A.) using 104 sera presented for routine pathological testing. The ELISAs were performed according to the manufacturers instructions (Table 1) with the serum diluent for the Gull and PanBio IgM tests containing goat anti-human IgG to remove competing IgG and rheumatoid factor. A positive result was defined as being positive in all three ELISAs. Discrepant sera were tested using a commercial IFA (MRL, U.S.A.), with IFA positivity defining a positive result. Similar criteria were used to determine a negative result.

The three VCA IgM ELISAs gave the same results in 94/104 (90%) of sera. The PanBio and Gull ELISAs showed the highest agreement (97%) and correlation (Pearson's $r = 0.93$, $p < 0.0001$) (Table 2, Figure 1d). All 3 assays gave high sensitivity when IFA was used to distinguish between the 10 discrepant test results. The Gull VCA IgM ELISA detected all 15 IgM positive specimens (sensitivity 100%), while the PanBio and Incstar VCA IgM ELISAs failed to detect 1/15 and 2/15 specimens respectively. The three assays also showed high specificity: 94% for Incstar and 98% for the PanBio and Gull ELISAs (Table 2).

The VCA IgG ELISAs also showed high agreement, though this was lower than observed with the IgM ELISAs. For example, the PanBio and Incstar tests showed discrepant results in 17 of 104 sera (overall agreement 84%) (Table 2). Variation between test values was evident when Pearson's correlation analysis was performed, with the highest correlation between VCA IgG values observed with the PanBio and Gull ELISAs ($r = 0.65$) (Figure 1a). The three VCA IgG ELISAs gave the same result in 85/104 (82%) of sera, and the ELISAs showed high sensitivity (>90%) when IFA was used to classify discrepant results (Table 2). However, the PanBio ELISA showed higher specificity (100%) than the Gull and Incstar VCA IgG ELISAs (73% and 47%), and the difference between specificity in the PanBio and Incstar ELISAs was significant (Fisher's Exact Test, $p = 0.0004$) (Table 2).

Differences between assay values and resulting sensitivity and specificity of the different assays is likely to be a reflection of the assay formats and antigens used by different manufacturers. The PanBio and Gull tests both use native VCA, while the Incstar test uses a synthetic peptide encompassing the major epitopes of the p18 protein. This protein is one of the major components of the VCA complex (Van Gransven *et al.*, 1994). The unglycosylated synthetic peptide antigen used in the Incstar ELISAs may be devoid of some epitopes since it would not contain carbohydrate antigens. Furthermore, it represents only part of the VCA complex. This may be the cause of the lower sensitivity observed in the Incstar IgM test, as observed in another study (Weidbrauk *et al.*, 1993). The PanBio and Gull tests showed a higher correlation and this is likely to reflect the use of similar native antigens. However, differences were still observed between these assays which may reflect differences in the native VCA preparations or methods used (e.g. blockers, diluents and plates). This was particularly evident with VCA IgG, where the PanBio ELISA showed perfect specificity (100%) while the Gull ELISA showed a specificity of only 73%. Furthermore, the Incstar ELISA showed poor specificity (47%), which may reflect the exposure of cross-reactive epitopes due to incorrect folding or exposure of cryptic non-specific epitopes masked in the native molecule by carbohydrate.

Table 1. Comparison Between Commercial EBV VCA ELISA Methods

Manufacturer	PanBio	Gull	Incstar
Assay format	Indirect ELISAs	Indirect ELISAs	Indirect IgG ELISA Capture IgM ELISA
Antigen	Purified native VCA (gp125)	Purified native VCA (gp125)	VCA p18 synthetic peptide
Serum	Diluted 1/100 (IgG & IgM) 20 min at 37°C	Diluted 1/21 (IgG) or 1/11 (IgM) 30 min at 37°C	Diluted 1/101 (IgG & IgM) 60 min at 37°C
Conjugate	Anti-human IgG or IgM HRP 20 min at 37°C	Anti-human IgG or IgM-alkaline phosphatase 30 min at 37°C	Anti-human IgG-HRP Anti-VCA p18-HRP 60 min at 37°C
Substrate	TMB 10 min	p-NPP 30 min	TMB 30 min
Stop solution	1M phosphoric acid	1.5N sodium hydroxide	1N sulphuric acid
Total incubation time	50 min	90 min	150 min

Abbreviations: VCA: Viral Capsid antigen; HRP: Horseradish peroxidase; TMB: Tetramethylbenzidine; pNPP: para-nitrophenol phosphate

Table 2. Performance of Commercial EBV VCA ELISAs

VCA IgM				
Manufacturer	Sensitivity	Specificity	Agreement	
PanBio	14/15 (93%)	87/89 (98%)	PanBio v Gull	101/104 (97%)
Gull	15/15 (100%)	87/89 (98%)	PanBio v Incstar	96/104 (92%)
Incstar	13/15 (87%)	84/89 (94%)	Incstar v Gull	96/104 (92%)
VCA IgG				
Manufacturer	Sensitivity	Specificity	Agreement	
PanBio	85/89 (96%)	15/15 (100%)	PanBio v Gull	93/104 (89%)
Gull	82/89 (92%)	11/15 (73%)	PanBio v Incstar	87/104 (84%)
Incstar	84/89 (94%)	7/15 (47%)	Incstar v Gull	94/104 (90%)

^a Fisher's Exact Test: $p > 0.05$ (non-significant) in all comparisons except between PanBio IgG specificity and Incstar IgG specificity ($p = 0.0004$).

Previous studies have reported good sensitivity and specificity for the Gull VCA IgM and VCA IgG ELISAs when Organon Technica and Gull IFA were used as the reference tests (Weidbrauk *et al.*, 1993; Gerber *et al.*, 1996). In the same study the Incstar VCA IgM ELISA did not perform as well relative to the Gull IFA (Weidbrauk *et al.*, 1993). The Incstar IgM ELISA showed poor sensitivity (53%) and high specificity (100%),

Figure 1: Comparison between ELISA values in (a) Gull and PanBio VCA IgG; (b) PanBio and Incstar VCA IgG; (c) Gull and Incstar VCA IgG; (d) Gull and PanBio VCA IgM; (e) PanBio and Incstar VCA IgM; and (f) Gull and Incstar VCA IgM. Assay values are represented on a log scale as a ratio of the test sample value to the cutoff sera value. The cutoff sera value (ratio = 1.0) is shown. Pearson's r-values, calculated after log transformation of data, are also shown. In all cases, $p < 0.0001$ when Pearson's correlation was performed.

compared to a sensitivity of 87% and specificity of 94% reported in this study. In this study the commercial IFA (MRL, USA) and ELISAs used were from different manufacturers. The VCA IgG IFA used a native VCA antigen, while the VCA IgM IFA used recombinant VCA expressed in a eukaryotic system. The VCA IgM antigen would be glycosylated, though differently to the native antigen. Consequently, the use of the MRL IFA would eliminate possible bias associated with using IFA and ELISAs from the same manufacturer. This was not the case in a previous study where Gull ELISA and IFA were compared (Gerber *et al.*, 1996).

In summary, commercial ELISAs for VCA IgM and IgG provide a simple and reproducible method for the serological diagnosis of infectious mononucleosis. However, the different tests can show variation in performance based on the different methods and reagents (including the antigens) used.

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