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Comparative performance of the Kalon and HerpeSelect enzyme-linked immunosorbant assays to determine the prevalence of herpes simplex virus type 2 in Papua New Guinea

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Abstract. ***Background*:** Infection with herpes simplex virus type 2 (HSV-2) is common worldwide and an important risk factor for HIV infection. Aetiological diagnosis of HSV-2 is typically determined with the use of commercially available type-specific enzyme-linked immunosorbent assays (ELISAs). This study aimed to determine the prevalence of HSV-2 among people attending sexual health clinics in the Highlands of Papua New Guinea. The study also aimed to compare the performance of two type-specific ELISA assays, the Kalon and HerpeSelect glycoprotein G2 assays, in this context. ***Methods*:** Participants were recruited as part of a longitudinal sexual health study. Participants attended four appointments over a 12-month period and had blood taken for HSV-2 serology at each time point. Both the Kalon and HerpeSelect assays were performed as per manufacturer's instructions. ***Results*:** A total of 132 participants were tested for HSV-2 using the Kalon and HerpeSelect ELISAs. HSV-2 prevalence was 52% (95% CI, 43–60) and 61% (95% CI, 52–69) with Kalon and HerpeSelect assays respectively. There was high concordance (87%, $\kappa = 0.75$, $P < 0.001$, $n = 115$) between the two assays at the manufacturer recommended index value cut-offs. For participants with discordant results at baseline, ($n = 16$), three sero-conversions were observed over the 12-month period when sequential sera was tested. ***Conclusions*:** A high HSV-2 prevalence was observed in this clinic-based population. Our longitudinal data indicate the higher prevalence of HSV-2 detected with the HerpeSelect ELISA was likely due to false positives rather than a higher sensitivity in the early stages of infection.

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Introduction

Herpes simplex virus type 2 (HSV-2) is primarily transmitted through sexual intercourse and is one of the most common sexually transmissible infections (STIs) in the world, with prevalence estimates varying between sex, age, geographic location and high-risk and non-high-risk populations.[1–3] A chronic, lifelong infection, HSV-2 has also been implicated in HIV transmission and acquisition and has been associated with adverse pregnancy outcomes.[2,3] In Papua New Guinea (PNG), there are no prevalence estimates from high-risk and/or clinic-based populations. The only HSV-2 prevalence estimates in PNG are from small community-based studies conducted over 10 years ago that reported a prevalence of 29.6% ($n = 54$) in a rural community in the south west of the country and 7.5% ($n = 93$) in the capital, Port Moresby.[4,5]

Accurate serological diagnosis of HSV-2 is important for disease control and prevention. The 'gold standard' diagnostic assays for HSV-2 detection, a western blot or a monoclonal antibody-blocking enzyme immunoassay (EIA), are expensive, labour intensive, operator dependent and generally not commercially available.[6] As a result, epidemiological studies of HSV-2 prevalence are heavily reliant on type-specific

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serological assays. The HerpeSelect glycoprotein G2 (gG2) ELISA (Focus Technologies, Cypress Hill, CA, USA) has been approved by the US Food and Drug Administration for the detection of IgG antibodies specific to HSV-2. Although validated for diagnostic purposes in the US, Europe and Australia, concerns have been raised about its performance, particularly in East African settings.[7–12] Another widely used assay, the Kalon gG2 ELISA (Kalon Biologicals, Aldershot, UK) has been shown to have an increased specificity and comparable sensitivity to the HerpeSelect assay in East African settings.[7,8–15]

Comparative performances of the Kalon and HerpeSelect gG2 ELISAs also vary by setting, by high-risk and non-high-risk populations and by reference testing methods used. Studies in China and Brazil have found both the Kalon and HerpeSelect assays to have specificities greater than 90%;[14,15] however, studies in Africa have generally observed a much lower specificity (40–80.6%).[7,8,11,16] Hypotheses to explain this lower specificity include cross reactivity with other unidentified antibodies, HIV infection and a greater sensitivity in the early stages of HSV-2 infection, thus potentially identifying more incident infections. A recent meta-analysis and systematic review pooled estimates of HerpeSelect and Kalon assay sensitivity and specificity from 35 studies from eight African countries and confirmed that not only did assay performance vary within Africa, but that it was consistently poorer than that reported in the US and Europe.[15]

To improve the specificity of the assays, researchers in previous studies have increased the recommended cut-off value for seropositivity from an optical density ratio of 1.1 to 3.5. This has been found to be effective, particularly for the HerpeSelect assay, although often at a cost to the sensitivity.[7–12,14] In studied African settings, the HerpeSelect assay performs optimally when scoring positive sera as those with an optical density index value of greater than 3.5,[16] whereas the Kalon assay performs optimally at an optical density value of between the manufacturer-recommended 1.1-fold and an increased value of 1.5-fold the cut-off calibrator.[8] There are no data on the optimal performance of HSV-2 serological assays in PNG.

A multi-disciplinary, community-based research program was conducted from 2007 to 2012 to investigate the acceptability, epidemiological impact and options for program implementation of male circumcision for HIV prevention in PNG. As part of this research, a longitudinal clinical cohort was established at two sites from 2010 to 2012 that included the determination of HSV-2 prevalence. As HSV-2 serology had never been validated for use in PNG, and considering the significant prevalence differences determined by different assays in East Africa, a sub-study was conducted to compare the performance of the two most widely used assays.

Methods

Participant recruitment and specimen collection

Informed consent was obtained from all participants. Following this, men and women attending two urban sexual health clinics in PNG [Tininga Clinic, Mount Hagen, Western Highlands Province (WHP) and Nine Mile Clinic, Port Moresby, National Capital District (NCD)] were enrolled into a longitudinal clinical cohort study. Participants had to be at least 18 years of age, and currently residing in either Mount Hagen or Port Moresby, with the intention of staying there for at least 12 months. Participants were asked to complete a face-to-face behavioural interview and undergo a clinical review, including a genital examination and the collection of laboratory specimens for STI diagnostics, at baseline, 12, 24 and 52 weeks following enrolment. Genital specimens for *Chlamydia trachomatis*, *Neisseria gonorrhoea* and *Trichomonas vaginalis* were tested by using real-time polymerase chain reaction assays (data not shown). A 10 mL venipuncture blood specimen was collected at baseline and clinical follow-up visits, and transported in batches to the PNG Institute of Medical Research (PNGIMR), Goroka, Eastern Highlands Province, for HSV-2 and syphilis testing (syphilis data not shown). All participants received at least one HIV test in the 12-month period. Diagnosed STIs were managed according to PNG national treatment guidelines. The recruitment period began in June 2010 and all follow-up visits were completed by August 2012.

Ethical approval was granted by all appropriate ethics committees including the PNGIMR Institutional Review Board, the PNG Medical Research Advisory Committee and the University of New South Wales Human Research Ethics Committee in Australia.

Laboratory procedures

Baseline testing for HSV-2 was performed by using the HerpeSelect (Focus Technologies) and Kalon gG2 (Kalon Biologicals) ELISAs, according to the respective manufacturers' instructions. Based on a previous literature review, the Kalon assay was selected as the standard test. These two assays are very similar 96-well indirect ELISAs that differ in terms of serum dilution factors (1 : 20 for Kalon and 1 : 101 for HerpeSelect) and enzyme substrates. For the HerpeSelect assay, sera was considered positive for HSV-2 antibodies if it gave an index optical density (OD) ratio greater than 1.1, and negative for HSV-2 antibodies with an index OD value less than 0.9. For the Kalon assay, sera giving OD results greater than 1.1-fold greater than that of the cut-off calibrator were considered positive and sera with an OD of less than 0.9-fold the OD of the cut-off calibrator were negative. For both assays, sera falling between what was considered positive and negative were considered equivocal. Results were also analysed with an increased positive cut-off OD ratio of 3.5 on the HerpeSelect assay, as this had been shown to increase the specificity in previous studies.

Samples with positive HSV-2 results as determined by Kalon assay at baseline were not re-tested at subsequent time points. All testing at 12, 24 and 52-week time points was undertaken using the Kalon assay only, and results were interpreted as outlined above. The test operator was blinded to the results obtained for the first assay. The laboratory at the PNGIMR is enrolled in an external quality assurance program for HSV-2 serology through the Royal College of Pathologists, Australia, and achieved satisfactory results for the period of this study.

Statistical analysis

The percentage agreement between the Kalon and HerpeSelect assays and their concordance was tested by using the Cohen's kappa coefficient (κ). We interpreted inter-rater agreement levels between the two assays as follows: 0.00–0.20 corresponded to slight agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement and 0.81–1.00 almost perfect agreement.[17] For positive and negative results only, the McNemar χ^2 test was used to explore the marginal homogeneity between HSV-2 results across the two assays. Contingency table analyses and Fisher's exact tests were used to compare proportions for independent samples. In all statistical tests, *P*-values less than 0.05 were considered statistically significant.

Results

A total of 153 participants aged between 18 and 53 years were enrolled into the longitudinal clinical cohort study; 69 from WHP and 84 from NCD. The median age of participants was 26 years (IQR: 11.5) and women constituted 55% ($n = 83$) of the study sample. A total of 132 participants had sera tested using both the HerpeSelect and the Kalon ELISAs at baseline. Sixty-eight (52%) sera were from participants recruited from WHP and 64 (48%) from NCD. At the respective manufacturers' recommendation for seropositivity, HSV-2 prevalence was 60.6% [95% confidence interval (CI), 52.1–68.5, $n = 80$] with HerpeSelect and 52.3% (95% CI, 43.1–59.9, $n = 69$) with Kalon. For WHP and NCD, HSV-2 prevalence was 60.3% ($n = 41$) and 60.9% ($n = 39$) respectively with HerpeSelect (Fisher's $P = 1.00$) and 54.4% ($n = 37$) and 50.0% ($n = 32$) respectively with Kalon (Fisher's $P = 0.66$). For females and males, HSV-2 prevalence was 75.3% ($n = 55$) and 42.4% ($n = 25$) respectively with HerpeSelect (Fisher's $P < 0.001$) and 68.5% ($n = 50$) and 32.2% ($n = 19$) with Kalon (Fisher's $P < 0.001$).

The overall concordance between Kalon and HerpeSelect assays at their manufacturer recommended index values was 87.1% ($n = 115$; Table 1) and the level of agreement between the assays as determined by the kappa analyses was 'substantial' and significantly greater than by chance alone ($\kappa = 0.75$, $P < 0.001$). Positive and negative concordances were 83% ($n = 68$) and 73.5% ($n = 47$) respectively. Despite the high level of agreement, there was bias with respect to discordant results across the two assays, such that the significant majority of discordant pairs were HerpesSelect positive and Kalon negative [McNemar's χ^2 (1) = 9.31, $P = 0.002$].

Table 1. Concordance between the Kalon IgG ELISA and HerpeSelect IgG ELISA at their manufacturer recommended cut-off index values of 1.1

ELISA, enzyme-linked immunosorbent assay. Results are presented as *n* values

		Kalon		
		Positive	Negative	Equivocal
HerpeSelect	Positive	68	12	0
	Negative	1	47	0
	Equivocal	0	4	0

Overall, 17 (12.9%) sera had discordant results between the two assays, of which 12 (70.6%) were positive by HerpeSelect and negative ($n = 12$) by Kalon (Table 1). Four (23.5%) participant samples were equivocal with HerpeSelect and negative on Kalon. A single (5.9%) sample was positive by Kalon and negative on HerpeSelect (Table 1). This patient's sequential sera samples were not tested, and thus is included in the Kappa analysis and marginal homogeneity test at baseline, but was excluded from the longitudinal analysis.

Increasing the assay cut-off index value to 3.5 at baseline resulted in a reduction of HSV-2 prevalence for both HerpeSelect (50% vs 60.6%) and Kalon (28.8% vs 52.3%) tests. Comparing HerpeSelect at an index value of 3.5 to Kalon at an index value of 1.1 gave an overall concordance of 85.6% ($n = 113$, Table 2) and the level of agreement between the assays remained relatively unchanged ($\kappa = 0.75$, $P < 0.001$). The negative concordance (73.5%, $n = 47$) did not change after increasing the index value, but there was a marked increase in equivocal results with HerpeSelect (1.1 index value: 3%, $n = 4$; 3.5 index value: 14%, $n = 18$; Fisher's $P = 0.003$) of which most (56%, $n = 10$) were positive on HerpeSelect and negative on Kalon at an index value of 1.1.

Of the 17 participants with discordant results at baseline (using an optical density ratio of 1.1), 16 had sequential samples tested (Table 3). By the 12-week visit, 12 out of 16 (75.0%) participants were sero-negative, two (12.5%) had sero-converted and two (12.5%) were lost to follow up (LTFU). By the 24-week visit, 10 out of 16 (62.5%) participants remained sero-negative; there were no additional sero-conversions and a total of four (25%) participants were LTFU. By the 52-week visit, six (37.5%) participants remained sero-negative, one (6.3%) participant sero-converted, giving a total of three (18.8%) sero-conversions, and seven (43.7%) participants were LTFU. At the completion of the study, 11 (68.8%) of these 16 participants with discordant results were sero-negative

Table 2. Concordance between the Kalon IgG ELISA at a cut-off index value of 1.1 and the HerpeSelect IgG ELISA at an increased cut-off index value of 3.5

ELISA, enzyme-linked immunosorbent assay. Results are presented as *n* values

		Kalon		
		Positive	Negative	Equivocal
HerpeSelect	Positive	65	1	0
	Negative	1	47	0
	Equivocal	4	14	0

Table 3. Longitudinal data for participants with discordant results [positive ($n = 12$) or equivocal ($n = 4$) on HerpeSelect, negative ($n = 16$) on Kalon] at baseline

Results are presented as counts with and percentages in parentheses

	Week 12	Week 24	Week 52
Negative	12 (75)	10 (62.5)	6 (37.5)
Positive	2 (12.5)	2 (12.5)	3 (18.8)
Lost to follow up	2 (12.5)	4 (25)	7 (43.7)
Total	16 (100)	16 (100)	16 (100)

for HIV, three (18.7%) participants declined a HIV test and two (12.5%) participants did not have their result recorded in the file.

Discussion

The baseline prevalence of HSV-2 infection was 52.3% with Kalon and 60.6% with HerpeSelect in our longitudinal clinical cohort study conducted among men and women attending two sexual health clinics in PNG. These are the first estimates of HSV-2 prevalence in this population and highlight the need for continued commitment to evidence-based public health interventions to improve sexual and reproductive health in PNG. Consistent with previous findings from other settings, HSV-2 prevalence was higher among the female participants compared with that among male participants.

In our study, the concordance between the Kalon and HerpeSelect assays was very high (87%) at their respective manufacturer recommendation for seropositivity. While previous investigations in Africa have been able to increase the specificity of the HerpeSelect assay by increasing the index value for the interpretation of a positive result,[7,13,16] we found that this substantially increased the number of equivocal results, and thus did not appear to improve the specificity of the HerpeSelect ELISA in our hands; rather, it added ambiguity to the results (Table 2).

It has previously been suggested that the HerpeSelect ELISA is more sensitive at detecting infections earlier in sero-conversion than either the Kalon ELISA or the western blot (WB) assay and that this may explain the lack of observed specificity with HerpeSelect in comparative studies. Ashley-Morrow *et al*.[18,19] demonstrated that for primary genital herpes episodes, the median time to seroconversion for the HerpeSelect assay was 21 days, for the Kalon assay it was 120 days and for the WB assay it was 87 days. The inclusion of the longitudinal data in this study therefore allows us to dispute the hypothesis that the higher number of infections detected by HerpeSelect is a function of increased sensitivity in the earlier stages of sero-conversion. A lack of symmetry of the discordant cases was evident, with most discordant results being HerpeSelect positive and Kalon negative. Tests of sequential samples of these discordant results with the Kalon assay revealed that at 24 weeks (168 days), two-thirds of discordant participants with a positive baseline HerpeSelect result, remained sero-negative and only one-sixth had sero-converted and the remaining quarter were lost to follow up; thus indicating that at least a proportion of the HerpeSelect results at baseline were likely due to false reactivity.

The high rate of loss to follow up is a significant limitation of this study. In addition, the lack of a comparison to a laboratory gold standard, such as WB, could be considered a limitation, as earlier comparisons of the Kalon and HerpeSelect assays have focussed on their performance against a reference WB assay and/or monoclonal antibody-blocking enzyme immunoassay, rather than a direct comparison[13–15] of the assays alone. Thus, although the inclusion of a comparison to a gold standard assay may have added clarity to the specificity of each assay, we felt that our data did not warrant the extra expense. We are able to conclude that the overall concordance of the assays was substantial and we did not observe the markedly different performance as has been reported in many East African studies. A further factor to consider when interpreting these results is the bias that could possibly have been introduced as a result of the recruitment for the parent trial, particularly around the strict criteria that required participants to be resident in the area for at least 12 months, and attend several appointments over the study period.

The high HSV-2 prevalence observed within this population is cause for concern. HSV-2 infection is recognised to be associated with both the sexual transmission and acquisition of HIV.[20,21] Currently, adult HIV prevalence in PNG is estimated at 0.9%. The two provinces in which this study was conducted, WHP and NCD, account for 20% and 22% of all reported notifications in the country respectively.[22] Baseline HIV prevalence in our cohort was 0% ($n = 54$) in WHP and 6% ($n = 84$) in NCD, and the prevalence of other STIs, including *Chlamydia trachomatis* (30%), *Neisseria gonorrhoea* (24%), *Trichomonas vaginalis* (18%) and syphilis (14%) were all high,[23] and consistent with earlier research in PNG.[24]

These are the first data on HSV-2 prevalence reported from clinic-based populations in PNG. On-going research by our group is providing the first robust general population estimates of HSV-2 prevalence in PNG, with results expected to be available in early 2015.[25] The comparative performance of HSV-2 type-specific serological assays indicates a lack of specificity with the HerpeSelect assay, and hence we recommend the use of the Kalon assay in future studies in PNG.

Conflicts of interests

None declared.

Author contribution

CR and AV conceived, designed and coordinated the study. CS conducted the laboratory work. CR, AV and CS conducted the literature review and drafted the manuscript. JA, JS, PK, ZK, PS and JK participated in the design and coordination of the study and helped draft and review the manuscript. PA led data analysis and conducted statistical tests and reviewed the manuscript. All authors read and approved the final manuscript.

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