CEPHIA

Consortium for the Evaluation and Performance of HIV Incidence Assays

Executive Summary/Conclusions

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Blood Systems Research Institute

Public Health England

Bill & Melinda Gates foundation

UCSF University of California San Francisco advancing health worldwide

SACEMA DST/NRF Centre of Excellence in Epidemiological Modelling and Analysis

The recommendations/ Conclusions within these reports are those of the CEPHIA group.

Copies of the reports and associated documentation can be obtained from the CEPHIA website.

http://www.incidence-estimation.org/page/cephia-overview

Enquiries

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Bio-Rad GS HIV-1/HIV-2 *PLUS O* EIA Avidity Assay

Executive Summary

Background

Monitoring the prevalence of HIV provides a blunt tool for understanding both recent transmission rates and the impact of behavioural changes or public health interventions on these rates. Consequently, there has been increasing application of assays, which are able to distinguish between 'recently' acquired HIV-1 infections and 'long-standing' infections, in cross-sectional surveys, to estimate HIV incidence. A comparative analysis of these existing incidence assays is a logical and necessary next step to facilitate the introduction of HIV incidence assays into wide use. In this assay, Bio-Rad GS HIV-1/HIV-2 *PLUS O* EIA Avidity Assay, we are using the test off-label and with a modified specimen procedure. The results presented here relate to the performance of the assay in a modified format and do not relate to the original U.S. FDA licensed HIV blood screening and diagnostic assay.

Evaluation Panel

The 'evaluation panel' consists of 2,500 uniquely-labelled HIV+ plasma specimens obtained from 928 distinct subjects, and was provided to laboratories in 5 sets of 500 specimens each. 75 of these specimens represent 25 aliquots of each of 3 underlying specimens, and acted as (unmarked) controls. Laboratory technicians were blinded to the specimen background information.

Data Analysis

The assay characteristics, namely the mean duration of recent infection (MDRI – average time 'recent' while infected for less than some time T) and false-recent rate (FRR – probability of a 'recent' result for an individual infected for longer than T), were estimated in a number of specimen sets. The MDRI (excluding treated subjects and identified elite controllers) is 333 days (95% CI: 302-363), for T=2 years and a Western blot HIV diagnostic test. The FRR in this specimen set is 6% (95% CI: 4-10%). High FRRs occur amongst treated subjects (50%), elite controllers (>10%) and virally suppressed subjects (>40%).

Technical Appraisal

This assay is widely available 3rd generation, HIV-1/HIV-2 antibody only test. It requires a skilled laboratory technician and lab equipment including incubator and plate reader. Plate washer is required to control the wash quality and reduce run to run variability. It also requires a stable electrical main supply but no additional electrical works. The assay is modified and requires a 1:10 dilution of the specimen, in either wash buffer or wash buffer containing DEA during the initial incubation step. There is no EQA programme for the modified assay.

Conclusions

This does not reach the Target Product Profile (TPP) for use in cross sectional incidence assays and we do not recommend its use alone.

Bio-Rad GS HIV-1/HIV-2 *PLUS O* EIA Avidity Assay Conclusion/Recommendations

The following conclusions relate to the assay in its off-label modified format and do not relate to its U.S. FDA licensed blood screening and diagnostic performance:

1) **Given its performance in relation to the Target Product Profile and to other candidate assays this assay is not recommended as a standalone assay for use in cross-sectional incidence assays.**

2) **In particular this assay is a not promising candidate assay due to its higher False Recent Rate.**

3) **However, the CEPHIA group believe that there is considerable scope for variation in cut-offs and thresholds used in this assay which along with currently described RITA algorithms (such as those including incorporating viral load, ARV treatment history, CD4 count or another assay) may improve the performance of this assay to an acceptable level for use in cross sectional incidence estimates.**

4) **This assay is a modified commercial assay and therefore serious consideration should be given by the user on how to control any manufacturing variation in this assay which may not be apparent in the assays licensed HIV blood screening and diagnostic use.**

5) **A number of groups are looking at these modified assays for a number of different purposes and alternative target product profiles are being prepared. The use of this assay for against other TPPs will be assessed when these are published.**

Ortho Avidity-VITROS ECi
Executive Summary

Background

Monitoring the prevalence of HIV provides a blunt tool for understanding both recent transmission rates and the impact of behavioural changes or public health interventions on these rates. Consequently, there has been increasing application of assays, which are able to distinguish between 'recently' acquired HIV-1 infections and 'long-standing' infections, in cross-sectional surveys, to estimate HIV incidence. A comparative analysis of these existing incidence assays is a logical and necessary next step to facilitate the introduction of HIV incidence assays into wide use. In this assay, the Avidity Vitros ECi , we are using the test off label and with a modified specimen. The results presented here relate to the performance of the assay in a modified format and do not relate to the licensed HIV diagnostic assay.

Evaluation Panel

The 'evaluation panel' consists of 2 500 uniquely-labelled HIV+ plasma specimens obtained from 928 distinct subjects, and was provided in 5 sets of 500 specimens each. 75 of these specimens represent 25 aliquots of each of 3 underlying specimens, and acted as (unmarked) controls. Laboratories were blinded to the specimen background information.

Data Analysis

The assay characteristics, namely the mean duration of recent infection (MDRI – average time 'recent' while infected for less than some time T) and false-recent rate (FRR – probability of a 'recent' result for an individual infected for longer than T), were estimated in a number of specimen sets. The MDRI (excluding treated subjects and identified elite controllers) is 285 days (95% CI: 254-316), for T=2 years and a Western blot HIV diagnostic test. The FRR in this specimen set is 7% (95% CI: 4-10%). High FRRs occur amongst treated subjects (>70%), elite controllers (>25%) and virally suppressed subjects (>60%).

Technical Appraisal

This assay is widely available 3rd generation, antibody only, assay performed on an automated platform. It requires a stable electrical mains supply but no additional electrical works. The platform can be used for a number of other assays and requires user and manufacturer maintenance to ensure optimal performance. The assay is simple to perform as it only requires a 1:10 dilution of the specimen, in either Phosphate Buffered Saline (PBS) or PBS containing 1M Guanidine, before testing following which all remaining steps are performed on-board the platform. The use of the automated platform ensures that the assay is very reproducible. There is no EQA programme for the modified assay nor analysis software for the modified format thus there is a possibility of operator error in avidity calculations.

Conclusions

This assay does not reach the Target Product Profile (TPP) for use in cross sectional incidence assays and we do not recommend its use.

Ortho Avidity-VITROS ECi
Conclusion/Recommendations

The following conclusions relate to the assay in its off-label modified format and do not relate to its licensed diagnostic performance:

1) **Given its performance in relation to the Target Product Profile and to other candidate assays this assay is not recommended for use in cross-sectional incidence assays.**

2) **In particular this assay is not a promising candidate assay due to its high False Recent Rate of 7%.**

3) **The CEPHIA group do not believe that varying of thresholds or use of currently described RITA algorithms will improve the performance of this assay to an acceptable level.**

4) **The automated nature of this assay means that there are considerable infrastructure demands for resource limited settings. However, the assay is extremely reproducible and the platform may be useful for other laboratory testing.**

5) **A number of groups are looking at these modified assays for a number of different purposes and alternative target product profiles are being prepared. The use of this assay for against other TPPs will be assessed when these are published.**

Ortho Less Sensitive (LS)-VITROS ECi
Executive Summary

Background

Monitoring the prevalence of HIV provides a blunt tool for understanding both recent transmission rates and the impact of behavioural changes or public health interventions on these rates. Consequently, there has been increasing application of assays, which are able to distinguish between 'recently' acquired HIV-1 infections and 'long-standing' infections, in cross-sectional surveys, to estimate HIV incidence. A comparative analysis of these existing incidence assays is a logical and necessary next step to facilitate the introduction of HIV incidence assays into wide use. In this assay, the LS-Vitros ECi, we are using the test off label and with a modified specimen. The results presented here relate to the performance of the assay in a modified format and do not relate to the licensed HIV diagnostic assay.

Evaluation Panel

The 'evaluation panel' consists of 2,500 uniquely-labelled HIV+ plasma specimens obtained from 928 distinct subjects, and was provided in 5 sets of 500 specimens each. 75 of these specimens represent 25 aliquots of each of 3 underlying specimens, and acted as (unmarked) controls. Laboratories were blinded to the specimen background information.

Data Analysis

The assay characteristics, namely the mean duration of recent infection (MDRI – average time 'recent' while infected for less than some time T) and false-recent rate (FRR – probability of a 'recent' result for an individual infected for longer than T), were estimated in a number of specimen sets. The MDRI (excluding treated subjects and identified elite controllers) is 306 days (95% CI: 274-338), for T=2 years and a Western blot HIV diagnostic test. The FRR in this specimen set is 10% (95% CI: 7-14%). High FRRs occur amongst treated subjects (>70%), elite controllers (>45%) and virally suppressed subjects (>60%).

Technical Appraisal

This assay is widely available 3rd generation, antibody only, assay performed on an automated platform. It requires a stable electrical mains supply but no additional electrical works. The platform can be used for a number of other assays and requires user and manufacturer maintenance to ensure optimal performance. The assay is simple to perform as it only requires a 1:400 dilution of the specimen in Ortho Diluent Buffer B before testing following which all remaining steps are performed on-board the platform. The use of the automated platform ensures that the assay is very reproducible. There is no EQA programme for the modified assay nor analysis software for the modified format thus there is a possibility of operator error in avidity calculations.

Conclusions

This does not reach the Target Product Profile (TPP) for use in cross sectional incidence assays and we do not recommend its use.

Ortho Less Sensitive (LS)-VITROS ECi
Conclusion/Recommendations

The following conclusions relate to the assay in its off-label modified format and do not relate to its licensed diagnostic performance:

1) **Given its performance in relation to the Target Product Profile and to other candidate assays this assay is not recommended for use in cross-sectional incidence assays.**

2) **In particular this assay is not a promising candidate assay due to its high False Recent Rate of 10%.**

3) **The CEPHIA group do not believe that varying of thresholds or use of currently described RITA algorithms will improve the performance of this assay to an acceptable level.**

4) **The automated nature of this assay means that there are considerable infrastructure demands for resource limited settings. However, the assay is extremely reproducible and the platform may be useful for other laboratory testing.**

5) **A number of groups are looking at these modified assays for a number of different purposes and alternative target product profiles are being prepared. The use of this assay for against other TPPs will be assessed when these are published.**

SEDIA™ HIV-1 LAg-Avidity EIA
Executive Summary

Background
Monitoring the prevalence of HIV provides a blunt tool for understanding both recent transmission rates and the impact of behavioural changes or public health interventions on these rates. Consequently, there has been increasing application of assays, which are able to distinguish between 'recently' acquired HIV-1 infections and 'long-standing' infections, in cross-sectional surveys, to estimate HIV incidence. A comparative analysis of these existing incidence assays is a logical and necessary next step to facilitate the introduction of HIV incidence assays into wide use.

Evaluation Panel
The 'evaluation panel' consists of 2,500 uniquely-labelled HIV+ve plasma specimens obtained from 928 distinct subjects, and was provided in 5 sets of 500 specimens each. 75 of these specimens represent 25 aliquots of each of 3 underlying specimens, and acted as (unmarked) controls. Laboratories were blinded to the specimen background information.

Data Analysis
The assay characteristics, namely the mean duration of recent infection (MDRI – average time 'recent' while infected for less than some time T) and false-recent rate (FRR – probability of a 'recent' result for an individual infected for longer than T), were estimated in a number of specimen sets. The MDRI (excluding treated subjects and identified elite controllers) is 188 days (95% CI: 165-211), for T=2 years and a Western blot HIV diagnostic test. The FRR in this specimen set is 1% (95% CI: 0-4%). High FRRs occur amongst treated subjects (>50%), elite controllers (>10%) and virally suppressed subjects (>45%).

Technical Appraisal
This assay is a commercially available assay developed specifically for the purpose of differentiating recent from long standing infections for use in studying cross-sectional studies. It is a manual EIA and requires apparatus available to most laboratories. There are two reagent packs one of which requires storage at -20^{o}C and the other at 4^{o}C. An EQA scheme and training in performance of the assay is available from CDC, Atlanta. Data management software for interpretation of the assay is available from the manufacturer. The assay is simple to perform following training.

Conclusions
This does not fulfil all components of the Target Product Profile (TPP) for use in cross sectional incidence assays. We do not recommend its use as a standalone assay but feel it may be useful as part of an Incidence assay algorithm.

SEDIA™ HIV-1 LAg-Avidity EIA
Conclusion/Recommendations –

1) **This assay does not reach all of the criteria of the Target Product Profile and therefore cannot be recommended for use alone for use in cross sectional incidence assays.**

2) **The performance of the assay when known confounders of assay performance are removed from the study population suggest that this assay may be usable as part of a testing algorithm in combination with clinical and other supporting information and potentially other incidence assays.**

3) **The CEPHIA group identified, in consultation with CDC, that performance of the assay can be improved by modifying the thresholds and this has been incorporated into the kit inserts by Sedia. As more data become available the CEPHIA group believe that a further review of the appropriate cut-offs be undertaken.**

4) **Following the change in cut-off used during this evaluation the CEPHIA group recommend that groups review their results and reanalyse their results using the new agreed cut-offs.**

5) **CEPHIA recognise and commend the work performed by CDC in strengthening the quality control around the worksheets used to calculate results and that these improvements have been adopted by SEDIA**

SEDIA™ BED HIV-1 Incidence EIA
Executive Summary

Background

Monitoring the prevalence of HIV provides a blunt tool for understanding both recent transmission rates and the impact of behavioural changes or public health interventions on these rates. Consequently, there has been increasing application of assays, which are able to distinguish between 'recently' acquired HIV-1 infections and 'long-standing' infections, in cross-sectional surveys, to estimate HIV incidence. A comparative analysis of these existing incidence assays is a logical and necessary next step to facilitate the introduction of HIV incidence assays into wide use.

Evaluation Panel

The 'evaluation panel' consists of 2,500 uniquely-labelled HIV+ plasma specimens obtained from 928 distinct subjects, and was provided in 5 sets of 500 specimens each. 75 of these specimens represent 25 aliquots of each of 3 underlying specimens, and acted as (unmarked) controls. Laboratories were blinded to the specimen background information.

Data Analysis

The assay characteristics, namely the mean duration of recent infection (MDRI – average time 'recent' while infected for less than some time T) and false-recent rate (FRR – probability of a 'recent' result for an individual infected for longer than T), were estimated in a number of specimen sets. The MDRI (excluding treated subjects and identified elite controllers) is 302 days (95% CI: 274-331), for T=2 years and a Western blot HIV diagnostic test. The FRR in this specimen set is 7% (95% CI: 4-11%). High FRRs occur amongst treated subjects (>60%), elite controllers (>15%) and virally suppressed subjects (>50%).

Technical Appraisal

This assay is a commercially available assay developed specifically for the purpose of differentiating recent from long standing infections for use in studying cross-sectional studies. It is a manual EIA and requires apparatus available to most laboratories. There are two reagent packs one of which requires storage at -25°C to -10°C and the other at 2 to 8°C. An EQA scheme and training in performance of the assay is available from CDC, Atlanta. Data management software for interpretation of the assay is available from the manufacturer. The assay is simple to perform following training.

Conclusions

This does not reach the current Target Product Profile (TPP) for use in cross sectional incidence assays and we do not recommend its use.

SEDIA™ BED HIV-1 Incidence EIA Conclusion/Recommendations

1) **The SEDIA™ BED HIV-1 Incidence EIA is a robustly reproducible assay that is easily deployed with technical ease. The assay is affordable and does not require significant additional laboratory equipment outside of that which could be reasonably expected.**

2) **However, given its performance in relation to the Target Product Profile, and to other candidate assays currently available, CEPHIA does not recommended it for use in cross-sectional incidence assays.**

3) **Although the assay does have dynamic range in which it can identify recent infection, CEPHIA feel that the false recent rate found in the evaluation of the assay does not make it a promising candidate for future widespread use.**

4) **A number of groups are looking at alternative uses for HIV Incidence assays and the potential exists that the assay may be better suited for some alternative uses. However, the CEPHIA group do not believe that varying of thresholds or application of currently described RITA algorithms will improve the performance of this assay to an acceptable level to support its inclusion cross sectional incidence studies. Evaluation of this assay against other TPPs will be performed when these are published.**

5) **CEPHIA has been led to believe that this assay may undergo a change in antigen at some time in the future. This may change the performance of the assay which CEPHIA is unable to evaluate. Should a change in antigen occur then this evaluation of the assay will no longer be relevant.**