Journal of Virological Methods 266 (2019) 114–120

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

Evaluation of a BioRad Avidity assay for identification of recent HIV-1 infections using dried serum or plasma spots

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A B S T R A C T

Serological methods to differentiate between recently acquired and established HIV-1 infections are a useful tool in the HIV-surveillance to characterize the epidemic, identify groups at risk and assess HIV-preventive interventions. Therefore, an avidity-based, modified BioRad Genscreen™ HIV-1/2 assay (BRA_{EUR}) was evaluated according to the avidity-based, modified BioRad HIV-1/2 Plus O protocol (BRA_{USA}). Overall, 692 well defined samples (82.5% B and 17.5% non-B subtypes) from recent (< 180 days, $n = 239$), intermediate (181–364 days, $n = 35$) or long term infections (≥365 days, $n = 419$) were used to determine a 'mean duration of recent infection' (MDRI), a 'median DRI' (MdDRI), the false recent rate (FRR), and concordance between the BRAs and the Sedia BED HIV-1 Capture enzyme immunoassay (BED). The optimal avidity index cut-off was determined to be 70% resulting in an MDRI of 233 days (95% IQR: 174–351) and an MdDRI of 171 days (95% IQR: 142–212). Concordance with the BRA_{USA} was high with 96.4%. The FRR of 6.0% as well as the MdDRI are similar to the BED (8.4%; 170 (139–214) days). Therefore, the BRA_{EUR} is a suitable alternative to replace the BED and trend analysis will be feasible after minimal adjustments for the MdDRI and the MDRI.

The surveillance of incident HIV infection is a valuable system to identify groups at risk and to assess the need or effectiveness of HIV-preventive interventions (ECDC, 2013; WHO, 2018). Therefore, a variety of serological assays (test for recency of infection, TRI) has been developed over the past twenty years in order to differentiate between recently acquired and established HIV-1 infections in cross-sectional studies (Barin et al., 2005; Duong et al., 2012; Janssen et al., 1998; Keating et al., 2012; Masciotra et al., 2010; Suligoi et al., 2002). Among those, the Sedia Biosciences BED HIV-1 Capture enzyme immunoassay (BED; Portland, Oregon, USA) (Parekh et al., 2002) was the first TRI that became commercially available and was therefore used worldwide (Hofmann et al., 2017; Kim et al., 2010; Scheer et al., 2009). In the following years avidity-based assays became established such as the modified BioRad HIV-1/2 Plus O assay (BRA_{USA}; Bio-Rad Laboratories, Redmond, WA, USA) (Masciotra et al., 2010) and the Limiting Antigen Avidity (LAg; Sedia™ HIV-1 LAg Avidity EIA; Sedia Biosciences Corporation, Portland, OR, USA) (Duong et al., 2012). These assays had shown improved performance by producing less 'false-recent' results (Kassanjee et al., 2014b). Therefore, the BRA_{USA} was implemented for the US national HIV surveillance system in 2016 (Hallett et al., 2009) and the LAg was implemented in cross sectional studies (Moyo et al., 2018) and for national HIV-incidence estimates (Soodla et al., 2018).

In 2008 the Robert Koch Institute implemented an HIV-1 incidence surveillance (Hofmann et al., 2017) based on the BED using blood residuals from newly diagnosed HIV-cases provided by diagnostic laboratories as dried serum or plasma spots (DSS or DPS) along with the mandatory anonymous report according to the German reporting system of notifiable diseases 'Protection against Infection Act §7 (3)'. In order to replace the BED in Germany, we decided to evaluated a TRI that should be easily accessible in Europe/Germany, reliable, applicable for dried serum spots (Masciotra et al., 2013) and for which previous testing was successful (Hauser et al., 2014). With regard to comparability of antibody assembly with the BioRad HIV-1/2 Plus O ELISA used for the BRA_{USA} the company referred to the BioRad Genscreen™ HIV-1/2 ELISA (Bio Rad, Marnes-la-Coquette, France). In the present study, we therefore report the evaluation of an avidity-based, modified BioRad Geenscreen™ HIV-1/2 assay (BRA_{EUR}) based on the BRA_{USA} protocol. Precisely characterized samples were used to determine the 'mean duration of recent infection' (MDRI, mean time that an individual is classified as recently infected), and the false recent rate (FRR; frequency of misclassified long-term infections as recent infection) as important parameters for the evaluation (Brookmeyer et al.,

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https://doi.org/10.1016/j.jviromet.2019.02.002
Received 29 November 2018; Received in revised form 1 February 2019; Accepted 6 February 2019
Available online 07 February 2019
0166-0934/

2013; Kassanjee et al., 2014a). Additionally, the median DRI (MdDRI) was calculated.

All specimens included in the present study were primary or follow-up samples collected from ARV-naïve individuals within the national multicenter long-term observational 'German HIV-1 Seroconverter (SC) Cohort' (Machnowska et al., 2019) with well-defined date of infection. Study patients are categorized as (i) 'acute SC' if the HIV-1 seroconversion is confirmed by laboratory diagnostics defined as reactive or undetermined ELISA followed by incomplete Immunoblot or detectable HIV-1 viral load or (ii) as 'documented SC' if a maximum interval of three years is given between the last negative and the first confirmed positive HIV-1 antibody test. For the here presented test evaluation the duration between the last negative and first confirmed positive test result was restricted to < 200 days for the recent infections. As date of infection either the blood sampling date for the first reactive test ('acute SC') or the arithmetic mean of the blood sampling dates for the last negative and first confirmed positive HIV-1 antibody test ('documented SC') was used. The duration of infection was calculated as the difference between the date of blood sampling and the date of infection. Patient characteristics such as sex, transmission mode, nationality, CD4 T-cell count, viral load and current ARV regimens are provided with the samples. Signed informed consent is obtained from all subjects prior to enrolment. The study protocol and the informed consent procedure was initially approved in 2005 by the Ethics Committee of the Charité, University Medicine Berlin (EA2/105/05), with approval amended and confirmed in 2013 (Zu Knyphausen et al., 2014).

The evaluation panel comprised 688 immunoblot positive specimens and four RNA positive but immunoblot negative specimens (692 totally) collected from 426 ARV-naïve individuals with 1–9 specimens per individual (mean: 1.6 specimen; median: 1 specimen, IQR 1–1) including 'acute SC' and 'documented SC' with a mean interval of 85 (IQR 65–145) days between the last negative and the first confirmed positive HIV-1 antibody test. Characteristics of the individuals and specimen included into the study are shown in Table 1. The mean age of the individuals at the first blood sampling date was 35 years (IQR 29–41). The median CD4 T-cell count and viral load at the first blood sampling date (or within 100 days thereafter) were 488 CD4 T-cells/ml (IQR: 379–646), and 60.100 viral copies/ml (IQR: 18.332–433.638), respectively. Duration of infection for each of the samples was ranging from 0 to 5929 days (mean: 772 days; median: 546 days, IQR: 56–1113). To calculate the FRR and false long term rate (FLTR) the panel was divided into three subsets: a 'recent infection sample set' including infection durations of ≤180 days (n = 239; 34.5%), an 'intermediate infection sample set' with infection duration between 181 and 364 days (n = 35; 5.1%) and a 'long term infection sample set' with ≥365 days (n = 419; 60.4%). These cut-offs were chosen due to the reported MDRI for BED and BRA_{USA} of 302 (274–331) and 333 days (302–363), respectively (Kassanjee et al., 2014b). The 'recent sample set' was mainly (207/239, 86.6%) selected from acute SC providing the most precise dates of infection. Samples of the 'long term sample set' were obtained to 62.1% (260/419) from acute SC. Due to HIV-1 subtype specific differences in the performance of TRIs (Kassanjee et al., 2014b), the composition of subtypes within the panel and the subsets was chosen to be an approximate to the German subtype distribution of 77% subtype B and 23% non-B subtypes (Hauser et al., 2017) shown in Table 2. FRR and FLTR was calculated for all subtypes, for subtype B, the total set of non-B subtypes and in particular for the most prevalent non-B subtypes in Germany subtype A, C and CRF02_AG (Hauser et al., 2017). CD4 T-cell counts below 200 cells/ml, as indicator for long term infections according to ECDC guidelines (ECDC, 2013), were reported for 9/419 (2.2%) samples from the long-term infection sample set but also for 9/239 (3.8%) samples from the recent infection sample set. In addition, low viral loads below a threshold of 75 copies/ml according to Kassanjee et al. (2016) as indicative for virally suppressed antiretroviral treated individuals and elite controllers were present in 6/419 (1.4%) samples in the long-term infection sample set and in 1/239 (0.4%) sample from the recent infection sample set with viral loads were ranging between 20 and 48 copies.

Due to the limited number of BRA_{USA} ELISA plates a reduced number of specimen randomly chosen from the evaluation panel (~50% recent and ~50% long term infections) was used as a 'comparison panel' (n = 357) in order to compare the concordance of both BRAs at different avidity index (AI) cut-offs ranging from 30% to 90% (increment of 20). It was composed of 174 samples with ≤180 days and 183 samples with ≥546 days duration of infection. The subtype distribution of the comparison panel is also shown in Table 2.

Plasma samples (100 µl) were dropped on filter cards (DPS), air dried and stored at −20 °C. Before application to TRIs, antibodies were eluted from DPS in 1000 µl or 500 µl elution buffer (500 ml PBS, 0.05% Tween, 3% FCS) resulting in a 1:10 or 1:5 dilution for the BED or both BRAs, respectively. The BED was performed according the manufactured instruction using the ODn ≤0.8 assay cut-off. Both BRAs were performed according to the two-well BRA_{USA} protocol provided by Masciotra et al. (Masciotra et al., 2010) using either 0.1 M diethylamine (DEA) dissociation agent or wash buffer. An AI was calculated from both OD values ($OD_{DEA}/OD_{wash\ buffer} \times 100$) for each sample. An AI cut-off of 30% was applied for the BRA_{USA} (Hanson et al., 2016). $OD_{wash\ buffer}$ values below 0.5 as a result of an ongoing seroconversion (low antibody concentration) were considered to be recent infections independent of the calculated AI.

The AI cut-off for the BRA_{EUR} was determined from the best accuracy (number of correctly classified samples) in the evaluation panel (AI 30%-90%, increment of 5). The MDRI and the MdDRI for the BRA_{EUR} as well as for the BED were calculated by adjusted mixed-effects logistic regression from the evaluation panel for all samples and for subtype B samples, exclusively. The MDRI allows to estimate the HIV-incidence using data from cross-sectional surveys (Brookmeyer et al., 2013). The MdDRI was calculated as an additional parameter to inform surveillance in a diagnosis based surveillance system in which the incidence cannot be estimated in a straightforward way. The fixed part of the model was described by Brookmeyer et al. (2013) and the random effects accounted for multiple samples contributed by the same individuals. Furthermore, the FRR was calculated from the number of specimen falsely classified as recent infection within the 'long-term infection sample set' and the FLTR was calculated from falsely classified specimen within the 'recent infection sample set'. BRAs and BED outcomes were compared with regard to FRR and FLTR and the distribution of the resulting population of recent infections.

Table 1
Characteristics of study population.

	Number of individuals		Number of samples	
	(n = 426)	%	(n = 692)	%
Sex				
Male	392	92.0	638	92.2
Female	34	8.0	54	7.8
Transmission risk				
MSM	365	85.7	595	86.0
HET	47	11.0	77	11.1
PWID	5	1.2	8	1.2
Not reported	9	2.1	12	1.7
Origin				
German	346	81.2	565	81.6
Non-German	80	18.8	127	18.4
SC category				
Acute SC	281	66.0	499	72.1
Documented SC	145	34.0	193	27.9
HIV-1 subtype				
B	346	81.2	571	82.5
Non-B	80	18.8	121	17.5

MSM: men who have sex with men; HET: persons with heterosexual mode of transmission; PWID: people who inject drugs; SC: seroconverter.

Table 2
Subtype distribution in the study panels in the total evaluation panel, the 'recent infection sample set' (≤180 days), the 'long term infection sample set' (≥365 days) and the comparison panel.

	Evaluation panel		'Recent' panel		'Long-term' panel		Comparison panel	
	N	%	*N*	%	*N*	%	*N*	%
Total	692		239		419		357	
Subtype B	571	82.5	193	80.8	349	83.5	305	85.4
Subtype A	22	3.2	8	3.3	14	3.3	10	2.8
Subtype C	24	3.5	8	3.3	13	3.1	10	2.8
Subtype D	2	0.3	2	0.8	0	0.0	0	0.0
Subtype F	5	0.7	4	1.7	1	0.2	0	0.0
Subtype G	5	0.7	2	0.8	3	0.7	4	1.1
CRF01_AE	18	2.6	7	2.9	11	2.6	10	2.8
CRF02_AG	31	4.5	8	3.3	21	5.0	18	5.0
Rare CRFs	3	0.4	1	0.4	1	0.2	0	0.0
URFs	11	1.6	7	2.5	6	1.2	0	0.0

Table 3
Assay characteristics for the total evaluation panel and for subtype B only calculated by adjusted mixed-effects logistic regression (a) for the BioRad Avidity at different AI cut-offs and (b) for the BED-CEIA at ODn cut-off ≤0.8.

(a) AI cut-off (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)	MdDRI (days)	Confidence interval (95%)	MDRI (days)	Confidence interval (95%)
All subtypes							
30	71.8	93.9	86.8	103	(80, 135)	173	(122, 254)
40	77.3	94.0	88.4	128	(102, 168)	201	(145, 292)
50	81.8	94.0	89.9	144	(116, 184)	201	(146, 301)
60	84.3	93.7	90.5	153	(127, 192)	209	(154, 318)
70	**87.0**	**92.8**	**90.7**	**171**	**(142, 212)**	**233**	**(174, 351)**
80	88.1	91.1	90.0	197	(164, 243)	284	(210, 407)
90	87.9	85.6	86.5	274	(223, 338)	393	(301, 488)
Subtype B							
30	73.5	95.3	88.3	102	(75, 148)	173	(122, 1061)
40	78.5	94.4	89.1	121	(94, 164)	204	(145, 644)
50	84.0	94.4	90.9	135	(107, 178)	196	(137, 589)
60	87.2	93.9	91.6	144	(118, 182)	190	(139, 302)
70	**90.1**	**93.2**	**92.2**	**164**	**(135, 206)**	**219**	**(162, 340)**
80	91.2	91.0	91.1	188	(155, 234)	271	(195, 399)
90	89.4	86.2	87.4	254	(204, 319)	383	(289, 482)

(b) ODn cut-off (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)	MdDRI (days)	Confidence interval (95%)	MDRI (days)	Confidence interval (95%)
All subtypes							
0.8	81.9	90.3	87.4	170	(139, 214)	184	(151–249)
Subtype B							
0.8	86.1	94.3	91.5	168	(129, 228)	172	(142–264)

MdDRI: median duration of recent infection, MDRI: mean duration of recent infection.

The optimal AI cut-off for the BRA_{EUR} was found to be 70% as determined from the evaluation panel with all subtypes as well as for subtype B only (Table 3a). Furthermore, this cut-off resulted in the highest concordance between the BRA_{EUR} and the BRA_{USA} with 96.4% (Table 4). Discordant results (n = 13/357; 3.6%) were partly due to FLT classification with either the BRA_{USA} (n = 2) or the BRA_{EUR} (n = 2) but mostly due to FR classifications by either BRA_{USA} (n = 6) or BRA_{EUR} (n = 3). Affected were subtype B (n = 8), subtype C (n = 1), CRF01_AE (n = 1) and CRF02_AG (n = 3).

Table 4
Concordance of the BioRad Avidity Europe versus USA at different BioRad Avidity Europe AI cut-offs while stable BioRad Avidity USA AI cut-off at 30% (n = 357).

BioRad Avidity Europe AI cut-off (%)	Concordance (*N*)	Concordance (%)
30	322	90.2
50	338	94.7
70	**344**	**96.4**
90	331	92.7

The MdDRI and the MDRI of the BRA_{EUR} at different AI cut-offs (30–90%) are shown in Table 3a. The MdDRI of 171 days (95% CI: 142, 212) for all subtypes and 164 days (95% CI: 135, 206) for subtype B was slightly shorter than the MDRI of 233 days (95% CI: 174, 351) for all subtypes and 219 days (95% CI: 162, 340) for subtype B (Table 3a, Fig. 1).

The FRR at AI cut-off 70% calculated from the long-term infection set (≥365 days of infection) was 6.0% (95% CI 4.0, 8.8) for all HIV-1 subtypes, 5.7% (95% CI 3.6, 8.9) for subtype B and 7.2% (95% CI 2.7, 16.8) for the non-B subtypes. The FLTR was 13.0% for all subtypes, 10.4% for subtype B, and 23.9% for the non-B subtypes (Table 5). The FRR and FLTR for the most prevalent non-B subtypes A, C and CRF02_AG and are shown in Table 5. However, sample counts were very low (n = 8–21) and confidence intervals therefore high.

None of the nine samples in the long-term infection set with a CD4 T-cell count below 200 cells/ml was falsely classified as a recent infection with the BRA_{EUR}. However, nine samples with CD4 T-cell counts below 200 cells/ml in the recent infection sample set would have been falsely reclassified as long term infections according to the ECDC and WHO guidelines. Since the infection duration is known here to be

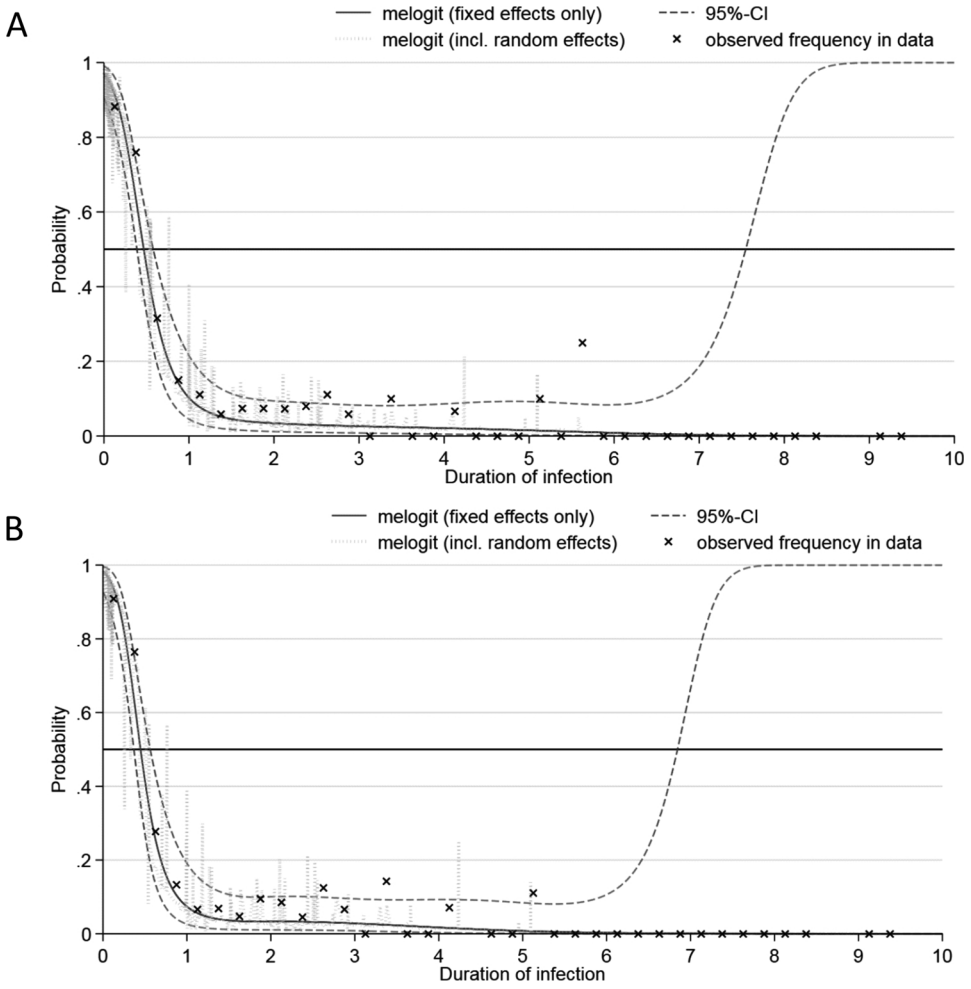

Fig. 1. Estimated probability for a positive AI test result as a function of the duration of the HIV infection estimated by the mixed effect logistic regression model. (A) For all subtypes, (B) for subtype B. Our data allowed estimating the probability for a recent infection for durations of infection up to 10 years. Since only few samples from patients with duration of infection longer than 7 years could be included, the confidence intervals are large for those durations.

recent, we refrained to do so. Furthermore, among the six samples with viral loads below 75 copies/ml in the long-term infection set two samples (33.3%) were falsely classified as recent infection. In contrast, the other four samples (66.7%) and the one sample in the recent infection set were correctly classified. This means in turn that confirmation of recent TRI-results with the additional viral load measurement above the 75 copies/ml threshold (Kassanjee et al., 2016) would result in two correct reclassifications in 'long term' but also one false reclassification of a true recent as a long term infection. Since the individuals of the present dataset are known to be ART-naïve, a reclassification was not carried out here.

The concordance of BRA_{EUR} and BED as calculated from the recent and long term dataset was 575/657 (87.5%) for all subtypes and 90.6% for subtype B (Table 6). Discordant results were mainly observed for the

Table 5
Estimated 'false recent rate' (FRR) and 'false long term rate' (FLTR) calculated from a 'long term' (> 52 weeks) and 'recent' infection sample set (< 26 weeks).

	Number of specimen	Falsely classified by BioRad EUR		Falsely classified by BED CEIA	
	(*N*)	(*N*)	% (95% CI)	(*N*)	% (95% CI)
FRR (%)					
All subtypes	**419**	**25**	**6.0 (3.9–8.7)**	**35**	**08.4 (5.9–11.4)**
Subtype B	**349**	**20**	**5.7 (3.5–8.7)**	**15**	**4.3 (2.4–7.0)**
Non-B subtypes	**69**	**5**	**7.2 (2.4–16.1)**	**20**	**28.9 (18.7–41.2)**
Subtype A	14	1	7.1 (0.2–33.9)	8	57.1 (28.9–82.3)
Subtype C	13	0	0.0 (0.0–24.7)	2	15.4 (1.9–45.4)
CRF02_AG	21	2	9.5 (1.2–30.4)	6	28.6 (11.3–52.2)
FLTR (%)					
All subtypes	**239**	**31**	**13.0 (9.0–17.9)**	**43**	**18.0 (13.3–23.5)**
Subtype B	**193**	**20**	**10.4 (6.4–15.6)**	**27**	**14.1 (9.4–19.7)**
Non-B subtypes	**46**	**11**	**23.9 (12.6–38.8)**	**16**	**34.8 (21.4–50.2)**
Subtype A	8	1	12.5 (0.3–52.7)	1	12.5 (0.3–52.7)
Subtype C	8	2	25.0 (3.2–65.1)	3	37.5 (8.5–75.5)
CRF02_AG	8	2	25.0 (3.2–65.1)	2	25.0 (3.2–65.1)

Table 6
Estimated discordance of the BED-CEIA and the BioRad Avidity Europe calculated from the 'long term' (≥365 weeks) and 'recent infection panel' (< 180 weeks) at an AI cut-off of 70%.

	Number of specimen (*N*)	Discordance (*N*)	Discordance (%)
All subtypes	657	82	12.5
Subtype B	554	52	9.4
Non-B subtypes	121	30	24.8
Subtype A	24	7	29.2
Subtype C	21	3	14.3
CRF02_AG	31	6	19.4

non-B subtypes (24.8%), mainly due to the higher FRR for non-B subtypes (particularly for subtype A) by the BED as compared to the BRA_{EUR} (Table 5).

The MdDRI of the BED (ODn cut-off ≤0.8) as calculated from the same evaluation panel is very similar to the MdDRI of the BRA_{EUR} with 170 days (95% CI: 139, 214) for all subtypes and 168 days (95% CI: 129, 228) for subtype B (Table 3b, Fig. 2). However, the MDRI of the BED (ODn cut-off ≤0.8) is shorter than the MDRI of the BRA_{EUR} with 184 days (95% CI: 151, 249) for all subtypes and 172 days (95% CI: 142, 264) for subtype B (Table 3b, Fig. 2).

Proportions of recent infections within the sub-groups sex, transmission risk and origin calculated from the recent infections data set and both TRIs are shown in Table 7. Non-significant differences were

Table 7
Characteristics of recent infected cases according to the reported time of infection and as estimated from the BED-CEIA and BioRad Avidity Europe classifications. Calculations are based on the recent and long term infection sample set.

	Recent infections		Recent infection according to BioRad EUR		Recent infection according to BED-CEIA	
	N	%	*N*	%	*N*	%
Total	239		233		231	
Sex						
Male	221	92.5	216	92.7	216	93.5
Female	18	7.5	17	7.3	15	6.5
Transmission risk						
MSM	205	85.8	200	85.8	210	90.9
HET	25	10.5	25	10.7	23	10.0
PWID	2	0.8	2	0.9	3	1.3
Not reported	7	2.9	6	2.6	4	1.7
Origin						
German	199	83.3	199	85.4	192	83.1
Non-German	40	16.7	34	14.6	39	16.9
HIV-1 subtype						
B	193	80.8	193	82.8	181	78.4
Non-B	46	19.2	40	17.2	50	21.6

MSM: men who have sex with men; HET: persons with heterosexual mode of transmission; PWID: people who inject drugs.

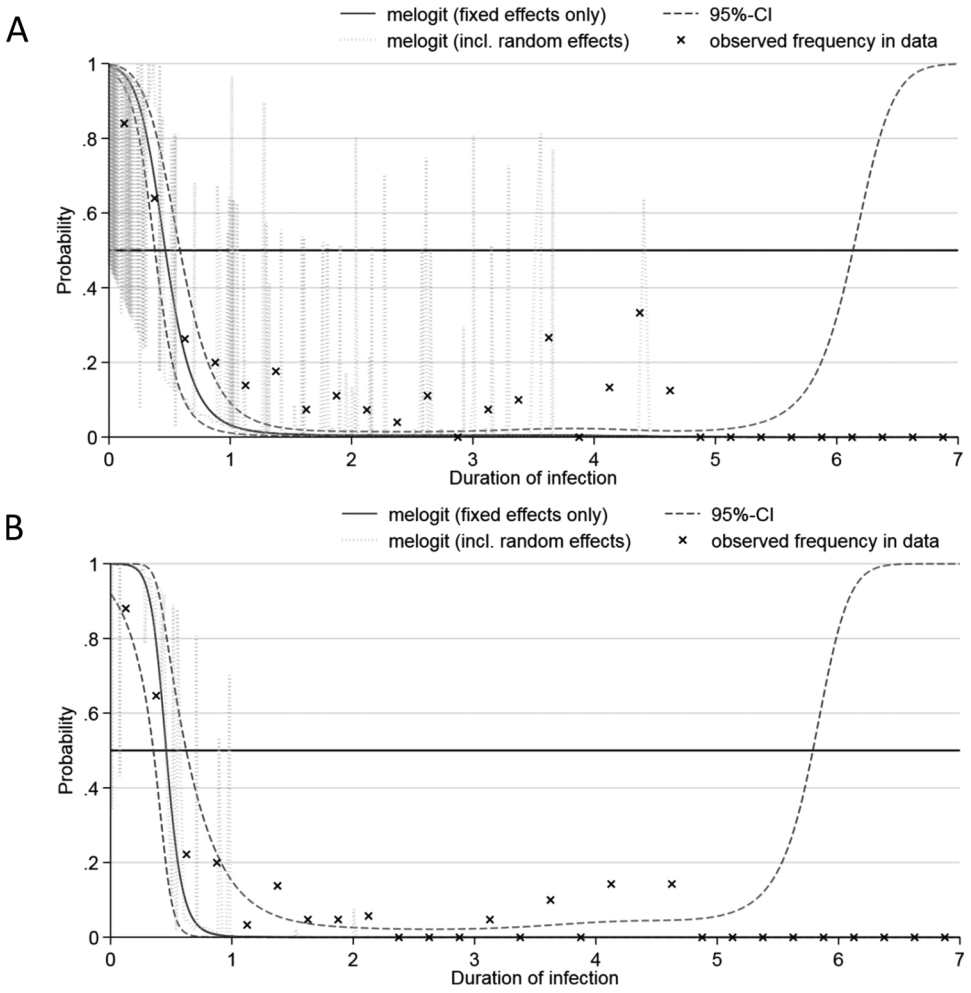

Fig. 2. Estimated probability for a positive BED result as a function of the duration of the HIV infection estimated by the mixed effect logistic regression model. (A) For all subtypes, (B) for subtype B. Our data allowed estimating the probability for a recent infection for durations of infection up to 7 years. Since only few samples from patients with duration of infection longer than 5 years could be included, the confidence intervals are large for those durations.

observed for the recent non-B infections which were overestimated by the BED while slightly underestimated by the BRA_{EUR} and for the proportion of non-Germans which were underestimated mainly by the BRA_{EUR} (Table 7).

We evaluated a TRI for its potential replacement of the BED, which was used in Germany from 2008 to 2018. Due to the similarity of the antibody assembly to the BRA_{USA} which was established for the national incidence screening in the USA (Hanson et al., 2016), an avidity-based, modified HIV-ELISA Genscreen HIV1/2 ELISA was chosen (BRA_{EUR}) and the protocol was adapted from the BRA_{USA} (Masciotra et al., 2010). An evaluation panel of HIV-1 infected individuals of known recent and long standing infections approximated to the German subtype distribution was used to calculate FRR, FLTR MdDRI and MDRI of the BRA_{EUR} and to analyze the concordance to the BRA_{USA} and comparability to BED. First, the optimal AI cut-off for the BRA_{EUR} was determined to be 70% for the subtype adapted sample set as well as for the most prevalent subtype B in Germany. For the BRA_{USA} a lower AI cut-off of 30% was reported (Masciotra et al., 2010). These different AI cut-offs might be a result of different antibody concentrations from DPS, lab specific procedures or other systematic differences which may affect the dissociation rate of the human antibodies from the plate coated proteins in the DEA reagent compared to that in wash buffer, finally resulting in stably higher AIs in our lab. However, the concordance between both BRAs applying the respective AIs was very high with 96.4%, pointing to an anti-antibody composition which was indeed very congruent in both BioRad ELISAs.

BRA_{EUR} performed with a FRR of 6.0% for all subtypes which is very similar to the published FRR of 6.2% for the BRA_{USA} reported by Kassanjee et al. (2014b) with an AI cut-off 40%. For subtype B the FRR was higher in our assay with 5.7% compared to the published rate of 2.1% (Kassanjee et al., 2014b). The MDRI calculated for our assay (233 days) was very similar to the BRA_{USA} with an MDRI of 239–254 days (Hanson et al., 2016). The MdDRI of the BRA_{EUR} (171 days) was generally shorter than the MDRI calculated for all subtypes as well as for subtype B.

Calculations for concordance, FRR and FLTR of BRA_{EUR} and BED were based on recent (< 180 days) and long term (≥ 365 days) infections sample sets to account for the published MDRIs (Hanson et al., 2016; Kassanjee et al., 2014b). They revealed slightly lower FRR and FLTR in the subtype mix for the BRA_{EUR} compared to the BED (6.0% vs 8.4% and 13.0% vs 18.0%, respectively). For subtype B alone the BRA_{EUR} performed with a slightly higher FRR (5.7% vs 4.3%) but with far lower FRR for non-B subtypes (7.2% vs 29.0%).

The FLTR was generally high in both assays with $\geq 10\%$. The FLTR is not reported by others in TRI evaluation studies and it seems that it is not considered to be an important parameter for TRIs which are used in cross sectional studies to estimate incidence. However, in Germany, where the proportion of recent and long term infections among newly diagnosed individual is approximately one to three, a systematic over- or underestimation of recent infections within specific HIV-positive groups might arise.

Concordance for BRA_{EUR} and BED was good with 87.5%. However, discordant classification mainly affected the non-B subtypes. Although the number of samples analyzed was low it seemed that particularly subtype A and CRF02_AG tended to be misclassified as false recent more frequently by the BED than by the BRA_{EUR}. A high FRR for subtype A by the BED was also shown in larger subtype A panels (Kassanjee et al., 2014b) or populations (Longosz et al., 2014). The CRF02_AG is a recombinant of subtype A and G with subtype A in the immune dominant region (envelope genomic region) which is crucial for the antibody binding. This seems to explain the high FRR of the BED for CRF02_AG. However, due to the limited number of specimen conclusions for specific non-B subtypes should be drawn with caution. The BED, designed as sandwich ELISA with recombinant proteins from subtype 'B', 'E' (CRF01_AE) and 'D' (Parekh et al., 2002), performed with a slightly lower FRR for subtype B than the BRA_{EUR}. Interestingly, the MdDRI of the BRA_{EUR} and the BED are very similar which will facilitate a trend analysis using the previous generated BED data and the new BRA_{EUR} data. According to ECDC incidence testing protocols (ECDC, 2013) CD4 T-cell counts below 200 cells/ml are indicating a long-standing infection. Thus, specimen with low AIs are recommended to be reclassified from 'recent' to 'long term'. However, a CD4 T-cell count below 200 cell/ml was also reported for 3.8% of the recently infected seroconverters in our evaluation panel, which – according to the guidelines – would be falsely reclassified into long term infections. CD4 T-cell counts might drop significantly in the first weeks of a new HIV-1 infection (Crum-Cianflone et al., 2009) therefore, this 200 cells/ml cut-off should be re-evaluated and a lower cell count might be more appropriate. In addition, very low viral loads in samples from long term infections indeed might result in false recent classifications. Therefore, application of thresholds for low or undetectable viral load as recommended by Kassanjee et al. (2016) seems to be appropriate for corrections.

To conclude, we successfully adapted the BRA_{USA} protocol to our BRA_{EUR} to replace the previously applied BED in the German HIV-incidence surveillance. The BRA_{EUR} is based on the easily available BioRad Geenscreen™ HIV-1/2 ELISA in Europe/Germany and is suitable for application on dried serum spots. Comparable MdDRIs of the BED and BRA_{EUR} resulting from our assay evaluation indicate that trend analysis using data from the previous BED and the new BRA_{EUR} will be feasible after minimal adjustments of MDRI differences.

Acknowledgments

We highly appreciate Dr. S. Michele Owen and Silvina Masciotra from the Centers for Disease Control and Prevention, Atlanta, GA, USA, for providing and sharing the optimized version of the BioRad Avidity assay protocol. We also thank to the participants of the German HIV-1 Seroconverter Study. This work was supported by the German Federal Ministry of Health [Grant number: FKZ: 2513AUK375].

References

Barin, F., Meyer, L., Lancar, R., Deveau, C., Gharib, M., Laporte, A., et al., 2005. Development and validation of an immunoassay for identification of recent human immunodeficiency virus type 1 infections and its use on dried serum spots. J. Clin. Microbiol. 43, 4441–4447.

Brookmeyer, R., Konikoff, J., Laeyendecker, O., Eshleman, S.H., 2013. Estimation of HIV incidence using multiple biomarkers. Am. J. Epidemiol. 177, 264–272.

Crum-Cianflone, N., Eberly, L., Zhang, Y., Ganesan, A., Weintrob, A., Marconi, V., Barthel, R.V., Fraser, S., Agan, B.K., Wegner, S., 2009. Is HIV becoming more virulent? Initial CD4 cell counts among HIV seroconverters during the course of the HIV epidemic: 1985–2007. Clin. Infect. Dis. 48, 1285–1292.

Duong, Y., Qiu, M., De, A., Jackson, K., Dobbs, T., Kim, A., et al., 2012. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. PLoS ONE 7, e33328.

ECDC, 2013. Monitoring recently acquired HIV infections in the European context. European Centre for Disease Prevention and Control, Stockholm.

Hallett, T.B., Ghys, P., Barnighausen, T., Yan, P., Garnett, G.P., 2009. Errors in 'BED'-derived estimates of HIV incidence will vary by place, time and age. PLoS ONE 4, e5720.

Hanson, D.L., Song, R., Masciotra, S., Hernandez, A., Dobbs, T.L., Parekh, B.S., Owen, S.M., Green, T.A., 2016. Mean recency period for estimation of HIV-1 incidence with the BED-Capture EIA and Bio-Rad avidity in persons diagnosed in the United States with subtype B. PLoS ONE 11, e0152327.

Hauser, A., Hofmann, A., Hanke, K., Bremer, V., Bartmeyer, B., Kuecherer, C., Bannert, N., 2017. National molecular surveillance of recently acquired HIV infections in Germany, 2013 to 2014. Euro Surveill. 22.

Hauser, A., Santos-Hoevener, C., Meixenberger, K., Zimmermann, R., Somogyi, S., Fiedler, S., Hofmann, A., Bartmeyer, B., Jansen, K., Hamouda, O., Bannert, N., Kuecherer, C., 2014. Improved testing of recent HIV-1 infections with the BioRad avidity assay compared to the limiting antigen avidity assay and BED Capture enzyme immunoassay: evaluation using reference sample panels from the German Seroconverter Cohort. PLoS ONE 9, e98038.

Hofmann, A., Hauser, A., Zimmermann, R., Santos-Hovener, C., Batzing-Feigenbaum, J., Wildner, S., Kucherer, C., Bannert, N., Hamouda, O., Bremer, V., Bartmeyer, B., 2017. Surveillance of recent HIV infections among newly diagnosed HIV cases in Germany between 2008 and 2014. BMC Infect. Dis. 17, 484.

Janssen, R., Satten, G., Stramer, S., Rawal, B., O'Brien, T., Weiblen, B., et al., 1998. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for

clinical and prevention purposes. JAMA 280, 42–48.
Kassanjee, R., McWalter, T.A., Welte, A., 2014a. Short Communication: Defining optimality of a test for recent infection for HIV incidence surveillance. AIDS Res. Hum. Retroviruses 30, 45–49.
Kassanjee, R., Pilcher, C.D., Keating, S.M., Facente, S.N., McKinney, E., Price, M.A., Martin, J.N., Little, S., Hecht, F.M., Kallas, E.G., Welte, A., Busch, M.P., Murphy, G., Consortium for the, E., Performance of, H.I.V.I.A., 2014b. Independent assessment of candidate HIV incidence assays on specimens in the CEPHIA repository. AIDS 28, 2439–2449.
Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA),Kassanjee, R., Pilcher, C.D., Busch, M.P., Murphy, G., Facente, S.N., Keating, S.M., Mckinney, E., Marson, K., Price, M.A., Martin, J.N., Little, S.J., Hecht, F.M., Kallas, E.G., Welte, A., 2016. Viral load criteria and threshold optimization to improve HIV incidence assay characteristics. AIDS 24, 2361–2371.
Keating, S.M., Hanson, D., Lebedeva, M., Laeyendecker, O., Ali-Napo, N.L., Owen, S.M., Stramer, S.L., Moore, R.D., Norris, P.J., Busch, M.P., 2012. Lower-sensitivity and avidity modifications of the vitros anti-HIV 1 + 2 assay for detection of recent HIV infections and incidence estimation. J. Clin. Microbiol. 50, 3968–3976.
Kim, A., McDougal, J., Hargrove, J., Rehle, T., Pillay-Van Wyk, V., Puren, A., et al., 2010. Evaluating the BED capture enzyme immunoassay to estimate HIV incidence among adults in three countries in sub-Saharan Africa. AIDS Res. Hum. Retroviruses 26, 1051–1061.
Longosz, A.F., Morrison, C.S., Chen, P.L., Arts, E., Nankya, I., Salata, R.A., Franco, V., Quinn, T.C., Eshleman, S.H., Laeyendecker, O., 2014. Immune responses in Ugandan women infected with subtypes A and DHIV using the BED capture immunoassay and an antibody avidity assay. JAIDS J. Acq. Imm. Def. 65, 390–396.
German HIV-1 Seroconverter Study Group,Machnowska, P., Meixenberger, K., Schmidt, D., Jessen, H., Hillenbrand, H., Gunsenheimer-Bartmeyer, B., Hamouda, O., Kücherer, C., Bannert, N., 2019. Prevalence and persistence of transmitted drug resistance mutations in the German HIV-1 Seroconverter Study Cohort. PLoS ONE 14 (1), e0209605.
Masciotra, S., Dobbs, T., Candal, D., Hanson, D., Delaney, K., 2010. Antibody avidity-based assay for identifying recent HIV-1 infections Based on Genetic Systems [TM] 1/2 Plus O EIA. In: 17th Conference on Retroviruses and Opportunistic Infections. San Francisco, CA. Poster#937.
Masciotra, S., Wei, X., Luo, W., Smith, A., Sionean, C., Paz-Bailey, G., Johnson, J., Owen, S.M., 2013. Performance of dried blood spots with a modified Genetic System [TM] HIV1/2 plus O antibody avidity-based assay for identifying recent HIV-1 infections. In: 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, GA. Poster#1055.
Moyo, S., Gaseitsiwe, S., Mohammed, T., Pretorius Holme, M., Wang, R., Kotokwe, K.P., Boleo, C., Mupfumi, L., Yankinda, E.K., Chakalisa, U., van Widenfelt, E., Gaolathe, T., Mmalane, M.O., Dryden-Peterson, S., Mine, M., Lebelonyane, R., Bennett, K., Leidner, J., Wirth, K.E., Tchetgen Tchetgen, E., Powis, K., Moore, J., Clarke, W.A., Lockman, S., Makhema, J.M., Essex, M., Novitsky, V., 2018. Cross-sectional estimates revealed high HIV incidence in Botswana rural communities in the era of successful ART scale-up in 2013-2015. PLoS ONE 13, e0204840.
Parekh, B., Kennedy, M., Dobbs, T., Pau, C., Byers, R., Green, T., et al., 2002. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. AIDS Res. Hum. Retroviruses 18, 295–307.
Scheer, S., Chin, C., Buckman, A., McFarland, W., 2009. Estimation of HIV incidence in San Francisco. AIDS 23, 533–534.
Soodla, P., Simmons, R., Huik, K., Pauskar, M., Jogeda, E.L., Rajasaar, H., Kallaste, E., Maimets, M., Avi, R., Murphy, G., Porter, K., Lutsar, I., Concerted Action on SeroConversion to, A, Death in Europe Collaboration in, E, 2018. HIV incidence in the Estonian population in 2013 determined using the HIV-1 limiting antigen avidity assay. HIV Med. 19, 33–41.
Suligoi, B., Galli, C., Massi, M., Di Sora, F., Sciandra, M., Pezzotti, P., et al., 2002. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. J. Clin. Microbiol. 40, 4015–4020.
WHO, 2018. WHO working group on HIV incidence measurement and data use. . Available at: http://www.who.int/diagnostics_laboratory/links/180622_boston_meeting_report.pdf?ua=1 [accessed 15.10.18].
Zu Knyphausen, F., Scheufele, R., Kucherer, C., Jansen, K., Somogyi, S., Dupke, S., Jessen, H., Schurmann, D., Hamouda, O., Meixenberger, K., Bartmeyer, B., 2014. First line treatment response in patients with transmitted HIV drug resistance and well defined time point of HIV infection: updated results from the German HIV-1 seroconverter study. PLoS ONE 9, e95956.