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Irish Blood Transfusion Service

Seirbhís Fuilaistriúcháin na hÉireann

PERFORMANCE EVALUATION OF ULTRIOPLEX E ASSAY

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Analytical sensitivity of the UltrioPlex E assay using different standards.

Can NAT detection limits based on different WHO replacement standards be compared?

IBTS

- IBTS NAT laboratory screens ~ 140,000 donations annually
- Procleix Ultrio Elite Multiplex assay for detecting HIV-1/2 RNA, HCV RNA, HBV DNA simultaneously. (2013)
- Procleix HEV Assay (2016)
- Both assays are performed as ID-NAT on the Grifols Procleix Panther System
- UE: Specificity 99.99%
- 2021; total tested 137,859, RRs-0 NRRs-12
- 2022; total tested 139,404, RRs-1 NRRs-13
- HEV: Specificity 99.96%
- 2021; total tested 137,859, RRs-39 NRRs-90 (10 S+).
- 2022; total tested 139,404, RRs-24 NRRs-53 (5 S+).

Overview of the UltrioPlex E assay

- The Procleix UltrioPlex E Assay (Grifols) was first commercially launched in August 2020 in Japan and received CE marking in 2021.
- The UltrioPlex E assay is a multi-target assay that combines the detection of HIV-1/2, HCV and HBV with HEV in a single, simultaneous test.
- The assay can be used with the Procleix Panther System.
- The Procleix UltrioPlex E assay differentiates HEV test results (“flasher signal”) from HIV, HCV and HBV (“glower signal”), however to determine if a positive sample is reactive for HIV, HCV and/or HBV additional discriminatory testing is required.

Assay Requirements

- Specificity: Negatives test Negative:
  - Impact: Repeat testing, donor impact, product loss
- Analytical Sensitivity: LOD: **95 % positive cut-off value** EU 2022/1107 **COMMON TECHNICAL SPECIFICATIONS FOR *IN VITRO* DIAGNOSTIC MEDICAL DEVICES**
- Clinical Sensitivity: identify patient samples with the virus
- Genotype detection:
  Comprehensive **genotype** coverage

Specificity of the Procleix assays according to the manufacturer's IFU

Specificity	Ultrio Elite HIV/HCV/HBV	Procleix HEV	UltrioPlex E HIV/HCV/HBV	UltrioPlex E HEV
Valid results	8011	2500	7948	7948
Initial Reactive	8	1	2**	0
True positive after repeat testing	0	0	1	0
Specificity after repeat testing	99.90 (99.80–99.95)	99.96 (99.77–99.99)	100 (99.95-100)	100 (99.95-100)

***One specimen was initially reactive (initial S/CO = 1.80) and repeat reactive (repeat test S/CO = 4.76) with the Procleix UltrioPlex E assay. The sample tested negative with anti-HBc serology. The specimen was designated as unresolved due to limited sample volume and was excluded from the specificity calculation.

Analytical sensitivity of the Procleix assays according to the manufacturer's IFU

WHO standard	Ultrio Elite & HEV assays 95% LOD (CI) IU/ml	WHO standard	UltrioPlex E assay 95% LOD (CI) IU/ml
HIV-1 97/650	18 (15.0 – 23.5)	**HIV-1 16/194**	38.2 (32.0-47.3)
HIV-2 08/150	10.4 (8.9 – 12.6)	**HIV-2 16/296**	9.5 (8.0 – 11.7)
HCV 06/100	3.0 (2.5 – 3.9)	**HCV 14/150**	9.6 (7.7 – 12.7)
HBV 97/750	4.3 (3.8 – 5.0)	**HBV 10/266**	3.1 (2.7 – 3.9)
HEV 6329/10	7.89 (6.6 -9.8)	**HEV 6329/10**	3.6 (3.1 – 4.5)

Analytical sensitivity of the Procleix assays according to the manufacturer's IFU

WHO standard	Ultrio Elite & HEV assays 95% LOD (CI) IU/ml	WHO standard	UltrioPlex E assay 95% LOD (CI) IU/ml
HIV-1 97/650 WHO 2nd IS	18 (15.0 – 23.5)	**HIV-1 16/194** WHO 4th IS	38.2 (32.0-47.3)
HIV-2 08/150	10.4 (8.9 – 12.6)	**HIV-2 16/296**	9.5 (8.0 – 11.7)
HCV 06/100 WHO 3rd IS	3.0 (2.5 – 3.9)	**HCV 14/150** WHO 5th IS	9.6 (7.7 – 12.7)
HBV 97/750	4.3 (3.8 – 5.0)	**HBV 10/266**	3.1 (2.7 – 3.9)
HEV 6329/10	7.89 (6.6 -9.8)	**HEV 6329/10**	3.6 (3.1 – 4.5)

IBTS UltrioPlex Study Design Clinical Performance

Specificity of the Procleix UltrioPlex E Assay

~5000 specimens from IBTS blood donors were tested using the UPxE assay.

Sensitivity of the Procleix UltrioPlex E Assay

- Known clinical positive specimens of 7 HIV, 6 HCV and 5 HBV and 34 HEV from IBTS blood donors retrospectively collected and stored at the IBTS were tested in UPxE

Analytical Performance

- Sensitivity Panels from Biologicals Quality Control B.V. (BioQControl) were used to compare the analytical sensitivity of the Procleix UltrioPlex E (UPxE) Assay and Procleix Ultrio Elite (UE) or Procleix HEV assays on the Panther system.
- **UltrioPlex E Assay: Operational Workflow Study**

IBTS UltrioPlex Study Design Clinical Performance Results

Specificity of the Procleix UltrioPlex E Assay

- 4997 IBTS blood donors samples tested. 1 NRR HEV on the UPxE. Specificity 99.98%

Clinical Sensitivity Procleix UltrioPlex E Assay

- All 18 positive donor samples of 7 HIV, 6 HCV and 5 HBV tested reactive on the UPX E assay and the relevant discriminatory assay
- All 34 HEV IBTS blood donors previous confirmed HEV reactives tested reactive with the UltrioPlex E assay
- **UltrioPlex E Assay:** Operational Workflow Study

Analytical Sensitivity

- The analytical sensitivity was determined for each viral marker using reference panels manufactured by BioQControl (the Netherlands). A minimum of 24 replicates were tested for each panel member. The 50% and 95% limits of detection (LOD) were calculated using probit analysis.
- BioQControl P0280 HBV-DNA genotype A
  - (calibrated on 1st and 2nd WHO standard for HBV)
- BioQControl P0288 HCV-RNA genotype 1
  - compared with the 1st and 2nd HIV International Standards in two WHO collaborative studies
- BioQControl P0290 HIV-1 RNA subtype B
  - calibrated against the 1st and 2nd WHO International Standards in the WHO collaborative study.
- BioQControl P0298 HIV-2 RNA subtype A
- BioQControl P0274 HEV-RNA genotype 3
  - (calibrated against the 1ST WHO 6329/10 standard for HEV RNA)

Panels tested to determine analytical sensitivity (BioQControl)

HIV-1	HIV-2	HCV	HBV	HEV
P0290 HIV-1 RNA subtype B	P0298 HIV-2 RNA subtype A	P0288 HCV-RNA genotype 1	P0280 HBV-DNA genotype A	P0274 HEV-RNA genotype 3
IU/ml	IU/ml	IU/ml	IU/ml	IU/ml
517	380.00	110	56.2	300
172	126.7	33	18.7	100
52	38.00	11	5.6	30
17	12.67	3.3	1.8	10
5.2	3.80	1.1	0.6	3
1.7	1.27	0.3	0.2	1
0.52	0.28	0.1	0.06	0.3
0.17	0.13	0.03	0.02	0.1

IBTS Results: BioQControl Analytical Sensitivity of the UltrioPlex E assay compared to the Ultrio Elite and HEV assays

	Ultrio Elite & HEV Assays		UltrioPlex E Assay	
	50% LOD (CI) IU/ml	95% LOD (CI) IU/ml	50% LOD (CI) IU/ml	95% LOD (CI) IU/ml
HIV-1	5.6 (4.1 – 7.6)	**23.2 (15.1 – 48.4)**	3.3 (2.3 – 4.7)	**21.3 (12.9 – 46.6)**
HIV-2	1.9 (1.3 – 2.7)	**13.8 (8.1 – 33.0)**	1.4 (1.1 – 1.95)	**6.4 (4.1 – 13.3)**
HCV	0.87 (0.6 – 1.3)	**7.3 (4.2 – 17.2)**	0.7 (0.5 – 1.1)	**5.5 (3.2 – 12.9)**
HBV	0.9 (0.7 – 1.4)	**7.3 (4.3 – 16.7)**	0.7 (0.5 – 1.0)	**5.1 (3.0 – 11.3)**
HEV	3.4 (2.5 – 4.5)	**11.4 (7.7 – 22.9)**	1.2 (0.8 – 1.8)	**9.2 (5.3 – 21.6)**

IBTS Results: BioQControl Analytical Sensitivity of the UltrioPlex E assay compared to the Ultrio Elite and HEV assays

	Ultrio Elite & HEV Assays		UltrioPlex E Assay	
	50% LOD (CI) IU/ml	95% LOD (CI) IU/ml	50% LOD (CI) IU/ml	95% LOD (CI) IU/ml
HIV-1	5.6 (4.1 – 7.6)	**23.2 (15.1 – 48.4)**	3.3 (2.3 – 4.7)	**21.3 (12.9 – 46.6)**
HIV-2	1.9 (1.3 – 2.7)	**13.8 (8.1 – 33.0)**	1.4 (1.1 – 1.95)	**6.4 (4.1 – 13.3)**
HCV	0.87 (0.6 – 1.3)	**7.3 (4.2 – 17.2)**	0.7 (0.5 – 1.1)	**5.5 (3.2 – 12.9)**
HBV	0.9 (0.7 – 1.4)	**7.3 (4.3 – 16.7)**	0.7 (0.5 – 1.0)	**5.1 (3.0 – 11.3)**
HEV	3.4 (2.5 – 4.5)	**11.4 (7.7 – 22.9)**	1.2 (0.8 – 1.8)	**9.2 (5.3 – 21.6)**

IBTS data for BioQControl reference panel for HIV

Ultrio Elite Assay		UltrioPlex E Assay	
50% LOD* (CI) IU/mL	95% LOD* (CI) IU/mL	50% LOD* (CI) IU/mL	95% LOD* (CI) IU/mL
6.73 (4.79-8.96)	**20.50** (14.75-34.16)	4.10 (2.86-5.60)	**15.23** (10.51-26.76)

HIV-1 P-0290 BioQC (IU/mL)	Ultrio Elite Assay		UltrioPlex E Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
517	24/24	100%	24/24	100%
172	24/24	100%	24/24	100%
52	24/24	100%	26/26	100%
17	21/24	88%	24/25	96%
5	12/24	50%	16/25	64%
2	2/24	8%	5/25	20%
1	0/24	0%	1/24	4%
0.2	0/24	0%	1/24	4%
0	0/24	0%	0/24	0%

*Calculated using Gompertz regression model (SAS 9.4)

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Grifols data for BioQControl reference panel for HIV

Ultrio Elite Assay		UltrioPlex E Assay	
50% LOD* (CI) IU/mL	95% LOD* (CI) IU/mL	50% LOD* (CI) IU/mL	95% LOD* (CI) IU/mL
6.76 (4.66-9.40)	**27.13** (18.18-50.76)	2.98 (2.08-4.15)	**10.93** (7.26-21.76)

HIV-1 P-0290 BioQC (IU/mL)	Ultrio Elite Assay		UltrioPlex E Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
517	24/24	100%	24/24	100%
172	24/24	100%	24/24	100%
52	24/24	100%	24/24	100%
17	20/24	83%	24/24	100%
5	10/24	42%	18/24	75%
2	3/24	13%	4/24	17%
1	2/24	8%	4/24	17%
0.2	0/24	0%	1/24	4%
0	0/24	0%	0/24	0%

*Calculated using Gompertz regression model (SAS 9.4)

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WHO International Standards

- Standardisation of NAT assays, meet regulatory requirements, improved sensitivity, specificity and allowed comparison studies.
- The first WHO IS for NAT assays, established in 1997, was HCV followed by HBV and HIV-1 in 1999
- Collaborative studies are carried out to establish an International Standard and include as many laboratories worldwide as possible.
- Biological materials of complex composition similar to actual clinical or blood donor specimens.
- A common biological unit of measurement, the International Unit (IU).
  - All other working reagents can then be calibrated against the International Standard and assigned a concentration in IU/ml.
- Lyophilised preparations with long-term stability.
- Adequate supply of the material (to last at least 5–10 years)

HIV WHO International Standards

- Laboratories, assay manufacturers use the WHO standards for validation of NAT methods
- WHO standards certain batch size (1000 to 2000 vials) requiring replacement after a number of years
  - Collaborative studies: calibrate the new standard against the preceding one
  - Potential for uncertainty in calibration in the WHO collaborative studies with new assays, new methods
  - May use different source material for preparation of the standards (introducing nucleic acid sequence differences)
  - Heat treatment of the standard and lyophilisation leading to matrix effects.

HIV WHO International Standards

HIV-1 International Standard	HIV-1 International Standard	Year	Unitage (IU/vial)
HIV-1 RNA 1st IS (97/656)	HIV-1 subtype B viremic human plasma diluted in pooled human cryosupernatant.	1999	100,000
HIV-1 RNA 2nd IS (97/650)	Cultured subtype B isolate diluted in pooled human cryosupernatant isolate distinct from 97/656).	2006	363,078
HIV-1 RNA 3rd IS (10/152)	Cultured and heat inactivated subtype B isolate (the same strain as 97/650) diluted in human plasma prepared as separate bulks from the same stock.	2011	185,000
HIV-1 RNA 4th (16/194)		2017	125,893

HIV WHO International Standards

- Drift in the amount of HIV-1 RNA per IU over the last two decades

  Lelie & Van Drimmelen. J Med Virol. 2020.
- Cross-calibration experiment,
- Significant drift in cp/IU of 19% with the third and 39% with the fourth WHO replacement standards as compared with the second WHO standard in the Abbott RealTime assay.
- VQC copy/IU conversion factor (95%CI) reduced by 39% from 0.41 (0.27-0.63) against the second to 0.25 (0.15-0.41) against the fourth WHO standard

.Analytical sensitivity of the Procleix assays according to the manufacturer's product inserts

WHO standard	Ultrio Elite assay 95% LOD (CI) IU/ml	WHO standard	UltrioPlex E assay 95% LOD (CI) IU/ml
HIV-1 97/650 WHO 2nd IS	18 (15.0 – 23.5)	**HIV-1 16/194** WHO 4th IS	38.2 (32.0-47.3)
Conversion Factor (Copies/IU)	x0.41		x0.25
Detection Probabilities (copies/mL)	7.38 (6.15-9.6)		9.6 (8-11.8)

Grifols Data: HIV-1 Analytical sensitivity with HIV-1 2nd WHO standard (97/650)

- A panel consisting of serially diluted HIV-1 2nd WHO standard (97/650) was used to evaluate analytical sensitivity with the Procleix UltrioPlex E and Ultrio Elite Assays.
- Estimations of 50% and 95% detection levels were determined by probit analysis using the Gompertz model.

Panel Tested	Procleix Assay	Detection Probabilities (IU/mL)	
		50% (95% Fiducial Limits)	**95%** (95% Fiducial Limits)
HIV-1 WHO (97/650)	UltrioPlex E	8.4 (5.3 – 10.9)	25.2 (18.8 – 45.4)
	Ultrio Elite	5.3 (2.0 – 8.0)	30.0 (20.3 – 70.0)

DISCUSSION

- Ultrio Elite and Ultrioplex assay results comparable, analytical sensitivity and specificity
- Value of using prepared sensitivity panel
  - Calibrated against WHO standards
  - >-80^{o}C storage
  - Ease of laboratory preparation and use
  - Gravimetrically prepared dilutions – Standardised preparation
  - Allow comparison of results across different assays, different platforms using the same standards, same concentrations
  - Allow comparison of results across different laboratories, manufacturers
- WHO standards
  - Potential for drift in concentration as new standards are produced.
  - WHO IS Like for like comparison required when comparing assays
  - Required as past of IVD

THANK YOU

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