Breach Candy Hospital Trust

TTI testing techniques: ELISA vs Chemiluminescence

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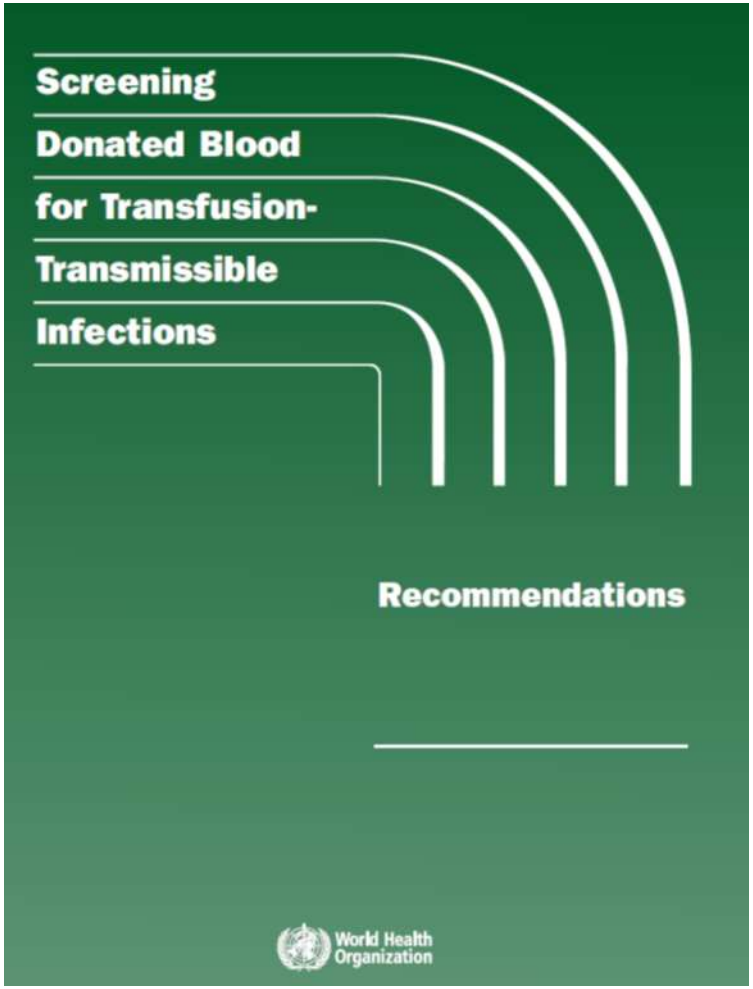
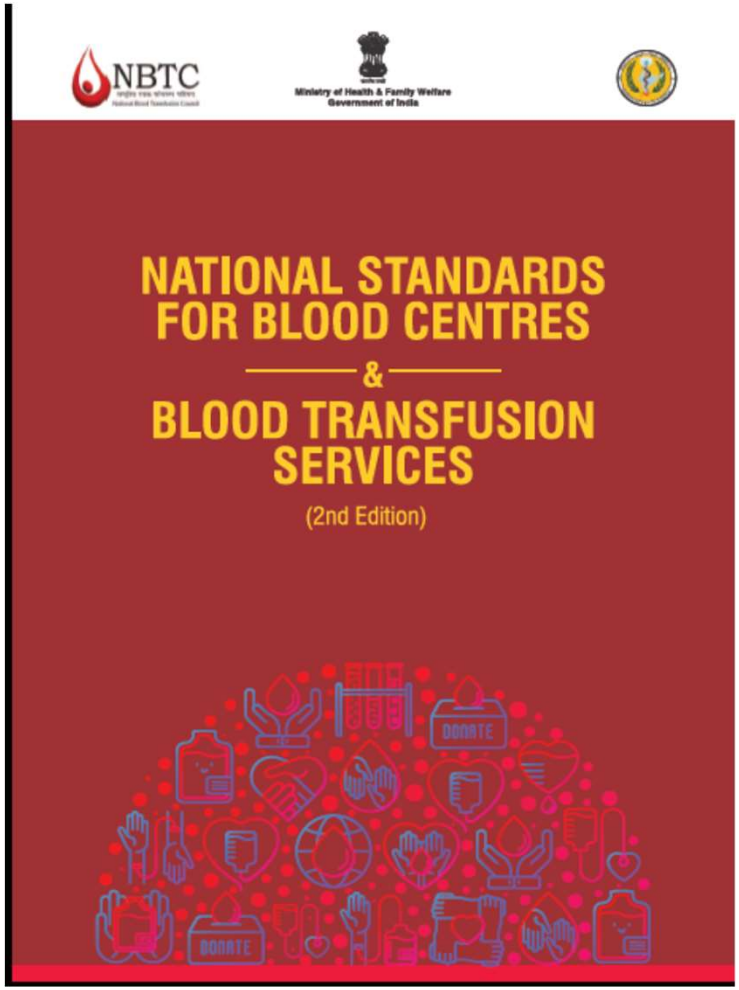
Overview

- Blood safety & TTIs
- Lab diagnosis of TTIs
- Recommended testing methods
- Literature review on ELISA vs CLIA

References


Screening
Donated Blood
for Transfusion-
Transmissible
Infections
Recommendations
World Health Organization


NBTC
Ministry of Health & Family Welfare
Government of India
NATIONAL STANDARDS
FOR BLOOD CENTRES
&
BLOOD TRANSFUSION
SERVICES
(2nd Edition)
DONATE
DONATE

Blood safety – basic premise

- Blood transfusion is life saving
- Risks associated : Infectious & non infectious
- Infectious complications : TTIs
- Mandate to ensure blood safety through effective screening programs
- Reduce the risk of TTIs to negligible

99.8% of the donations in high-income countries and 99.9% in upper-middle-income countries are screened following basic quality procedures, as compared to 83% in lower-middle-income countries and 76 % in low-income countries

Table 1. Prevalence of transfusion-transmissible infections in blood donations (Median, Interquartile range (IQR)), by income groups

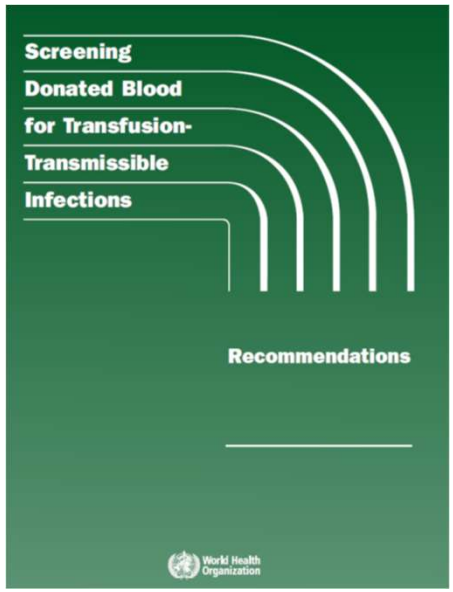
	HIV	HBV	HCV	Syphilis
High-income countries	0.002% (<0.001% – 0.01%)	0.02% (0.005% – 0.12%)	0.007% (0.002% – 0.06%)	0.02% (0.003% –0.12%)
Upper middle-income countries	0.10% (0.03% – 0.23%)	0.29% (0.13% – 0.62%)	0.19% (0.07% – 0.36%)	0.35% (0.13% –1.10%)
Lower middle-income countries	0.19% (0.04% – 0.62%)	1.70% (0.70% – 4.74%)	0.38% (0.12% –0.99%)	0.69% (0.19% – 1.38%)
Low-income countries	0.70% (0.28% – 1.60%)	2.81% (2.00% – 6.02%)	1.00% (0.50% – 1.67%)	0.90% (0.60% – 1.81%)

WHO Global Database on Blood Safety from 108 countries for the year 2018

www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability

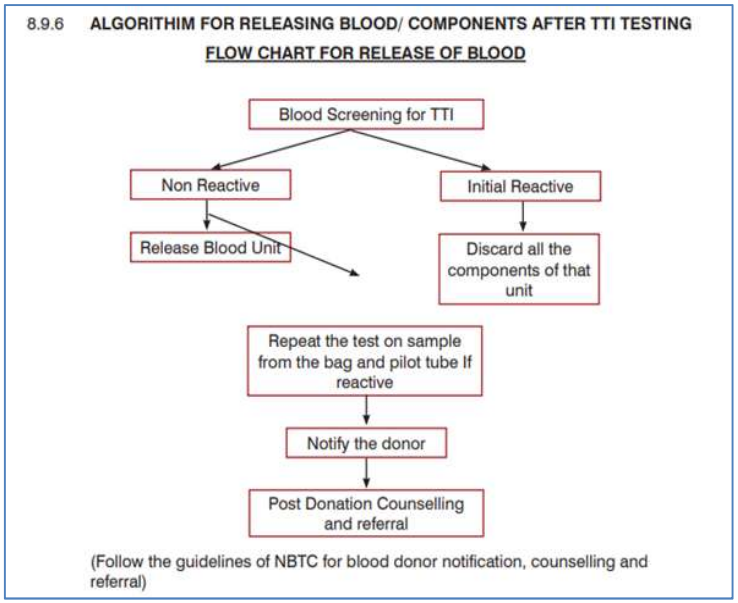
Transfusion Transmissible infections

- Human immunodeficiency virus (HIV)
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- *Treponema pallidum* (Syphilis)
- Plasmodium species (Malaria,India)



Laboratory diagnosis

- HIV-1 and HIV-2: antigen-antibody or HIV antibodies
- Hepatitis B: surface antigen (HBsAg)
- Hepatitis C: antigen & or antibody
- Syphilis (*Treponema pallidum*): specific treponemal antibodies or non specific tests
- Malaria : antigen



Source : WHO

Technical specifications - WHO

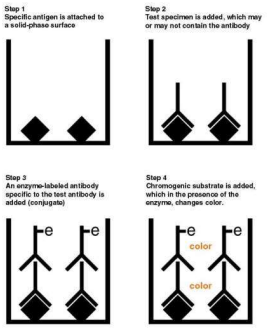
- Minimum evaluated Sn & Sp levels of all assays used for blood screening should not be < 99.5%

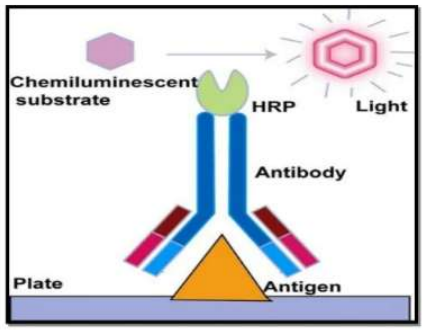
Assay presentation
Clarity of instructions
Ease of use
Assay characteristics, including Sn/Sp
Sample volume
Sample and reagent addition monitoring
Robustness
Assay reproducibility and precision
Number of tests per assay
Kit size
Total assay time

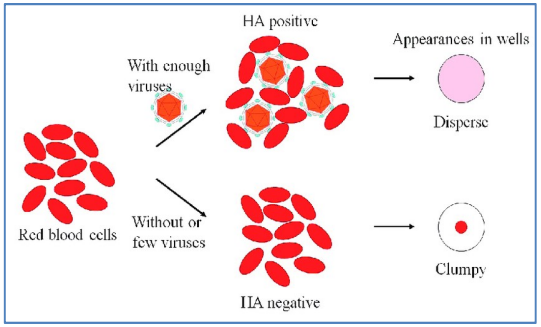
TTI testing methods

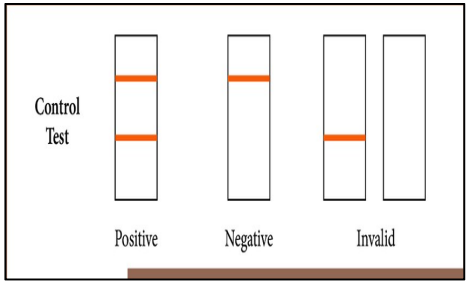
The main types of assay used for blood screening are:

- Immunoassays (IAs):
  - Enzyme immunoassays (EIAs)
  - Chemiluminescent immunoassays (CLIAs)
  - Haemagglutination (HA)/particle agglutination (PA) assays
  - Rapid/simple single-use assays (rapid tests)
- Nucleic acid amplification technology (NAT) assays.









Immunoassays(IAs)

- Detect antibody, antigen or both
- Enzyme and chemiluminescent IAs are currently the most commonly
- Differ only in the mode of detection of immune complexes
- ELISA : color generation is measured
- CLIA : light produced due to the chemical reaction is measured

LITERATURE REVIEW

The better screening tool :
ELISA vs CLIA

Comparative evaluation of ELISA & CLIA screening assays in the effective detection of HIV infection in blood donor samples: An observational study from a blood bank in tertiary health center

- Out of 850 samples, 98 were confirmed HIV positive by qPCR
- Similar sensitivity
- CLIA showed a higher specificity rate (CLIA: 99.6%, 749/752)
- Higher PPV (CLIA: 94.4%, 92/98) ($P < 0.05$)
- Better concordance rate (CLIA:99.2%, 843/850)
- CLIA's kappa value was the highest among all the serologic assays

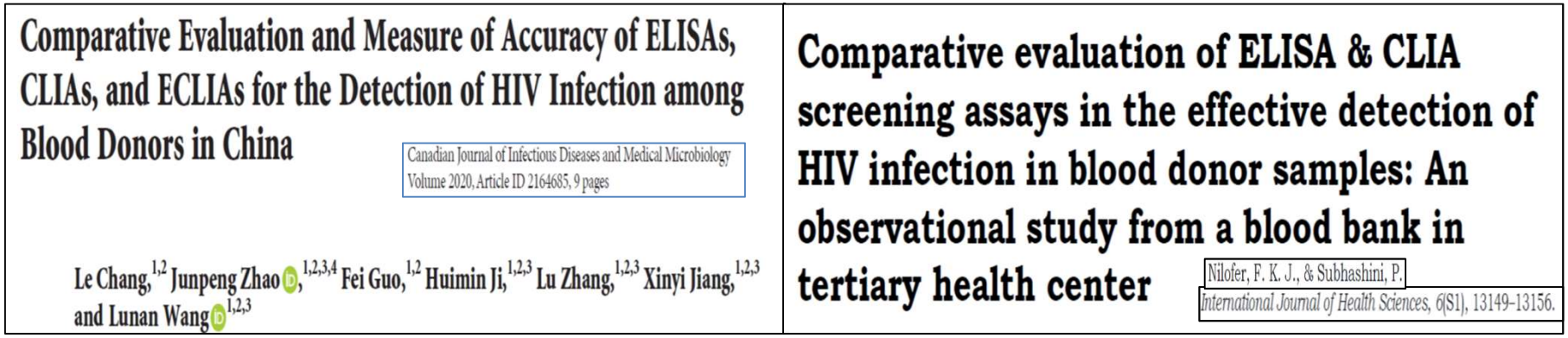
Nilofer, F. K. J., & Subhashini, P. (2022). *International Journal of Health Sciences*, *6*(S1), 13149–13156

Comparative Evaluation and Measure of Accuracy of ELISAs, CLIAs, and ECLIAs for the Detection of HIV Infection among Blood Donors in China

Canadian Journal of Infectious Diseases and Medical Microbiology
Volume 2020, Article ID 2164685, 9 pages

Le Chang,[1,2] Junpeng Zhao,[1,2,3,4] Fei Guo,[1,2] Huimin Ji,[1,2,3] Lu Zhang,[1,2,3] Xinyi Jiang,[1,2,3] and Lunan Wang[1,2,3]

- ✓ 14 blood centers: multicentric study
- ✓ 1029 samples, 136 HIV positive
- ✓ CLIA Sp : 99.1%
- ✓ Concordance rate 99.2%
- ✓ PPV CLIA:94.4% ($P < 0.05$)
- ✓ Kappa values of CLIA 0.967



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Conclusion:

- Compared with ELISA, CLIA is more specific and accurate in detecting HIV antibody/antigen
- CLIA can be used for the improvement of serological blood screening strategy
- Avoids the unnecessary loss of blood donors

Original Research Article DOI: 10.18231/2394-5478.2017.0078

Comparison between ELISA and chemiluminescence immunoassay for the detection of Hepatitis C virus antibody

Pampi Majumder[1], Anup Kumar Shetty[2,*]

[1]PG Resident, [2]Associate Professor, Dept. of Microbiology, Father Muller Medical College, Mangalore, Karnataka

- Observational cross-sectional study
- 91 samples included
- ELISA technique as gold standard
- 2(6.25%) CLIA reactive samples were found to be non-reactive by ELISA
- 29 (31.87%) samples were interpreted as borderline on CLIA
- CLIA : Sp : 96.07% specificity and Sn: 96.66%

Indian J Microbiol Res 2017;4(4):353-357

Performance Evaluation of Blood Donor Screening Assays for Serological Detection of Hepatitis B Surface Antigen and Antibodies to Hepatitis C Virus

Meenu Bajpai[2], Brinda Kakkar[1], Ekta Gupta[2], Guresh Kumar[2]

How to cite this article: Bajpai M, Kakkar B, Gupta E, Kumar G. Performance evaluation of blood donor screening assays for serological detection of hepatitis B surface antigen and antibodies to hepatitis C virus. Glob J Transfus Med 2021;6:205-10.

1000 consecutive blood donors screened

ELISA was the reference standard

Table 4: Performance of rapid diagnostic tests and chemiluminescence immunoassay against enzyme-linked immunosorbent assay

Infectious marker detected	Type of assay	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	FP* (*n*)	FN* (*n*)	Youden's weighted index
HBsAg (*n*=1000)	RDT_1	64.29	100	100	99.5	0	5	64.29
	RDT_2	64.29	99.9	90	99.49	1	5	94.29
	CLIA	71.43	97.77	31.25	99.59	22	4	69.2
Anti-HCV (*n*=1000)	RDT_3	25	100	100	99.7	0	3	25
	RDT_4	25	100	100	99.7	0	3	25
	CLIA	75	99.7	50	99.9	3	1	74.7

*As confirmed by PCR. RDT: Rapid diagnostic tests, CLIA: Chemiluminescence immunoassay, HbsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus, PPV: Positive predictive value, NPV: Negative predictive value, FP: False positive, FN: False negative, PCR: Polymerase chain reaction

Conclusions: Performance of CLIA as screening assay was better compared to RDTs. CLIA seems to be a suitable screening assay for emergency situations and predonation apheresis donor screening. RDTs may be used as supplemental assay prior to donor notification.

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

Head-to-head comparison of Enzyme Linked Immunosorbent Assay (ELISA) and Enhanced Chemiluminescence Immunoassay (ECLIA) for the detection of Transfusion Transmitted Disease (TTD) Markers; HIV, HCV and HBV in blood donors, in India

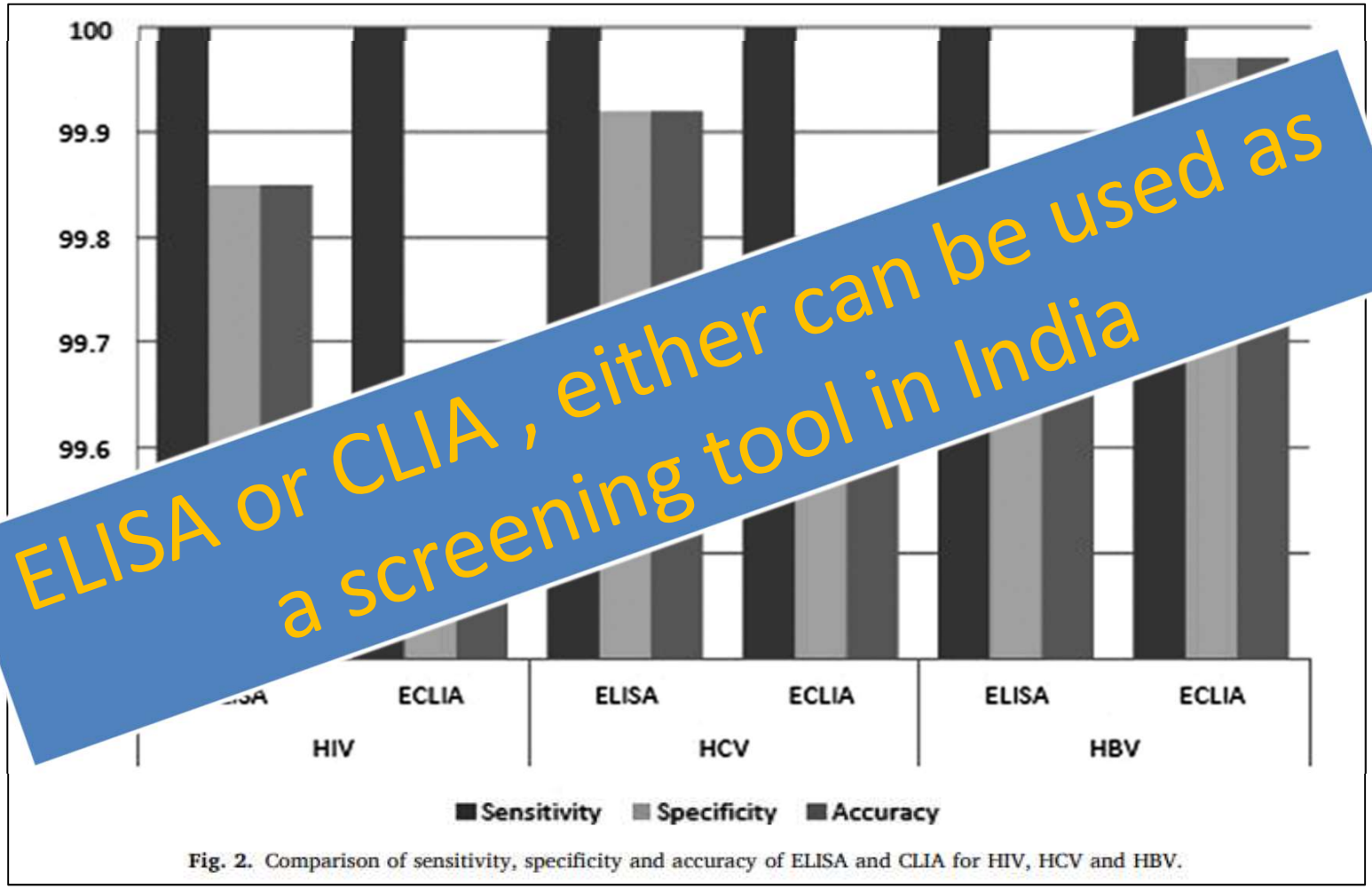
Aseem Kumar Tiwari, Anand Prakash Upadhyay *, Dinesh Arora, Tina Wadhwa, Geet Aggarwal, Swati Pabbi, Aanchal Sunil Luthra, Sunder Singh Rawat

Medanta- The Medicity Hospital, Department of Transfusion Medicine, Sector-38, Gurgaon, India

Table 1
Sensitivity, specificity, antigens and antibodies coated on solid phase of assays used in ELISA and ECLIA for detection of TTD markers (as per manufacture's kit insert).

TTD	HIV		HCV		HBV	
Testing Method	ELISA	ECLIA	ELISA	ECLIA	ELISA	ECLIA
Assay	Genscreen™ ULTRA HIV Ag-Ab assay	VITROS HIVc	Monolisa™ HCV ULTRA V2	VITROS aHCV	Monolisa™ HBsAg ULTRA	VITROS HBsAg
Sensitivity %	100	100	100	100	100	100
Specificity %	99.95	99.84	99.94	99.76	99.94	99.98
Antigens and antibodies coated on solid phase	Monoclonal anti-HIV p24 Ab, purified Antigens gp160 (envelop) recombinant protein, HIV-1 group O-specific epitope and peptide mimicking immunodominant epitope of HIV-2	Biotinylated anti-HIV p24 Ab, HIV–1 envelope (env 13), HIV-1 group O envelope (env 70–3) and HIV–2 envelope (env 31).	Capsid peptide NS3, NS4	C22–3, NS3, NS4, NS5	Monoclonal anti-HBs antibodies	Monoclonal anti-HBs antibodies

- ✓ ELISA vs CLIA comparison of 10164 samples
- ✓ Gold standard : Individual Donor-Nucleic Acid Amplification Test (ID-NAT)



ELISA or CLIA , either can be used as a screening tool in India

Fig. 2. Comparison of sensitivity, specificity and accuracy of ELISA and CLIA for HIV, HCV and HBV.

Is there a real advantage of one over the other?

Technical specifications - WHO

- Minimum evaluated Sn & Sp levels of all assays used for blood screening should not be < 99.5%

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Number of tests per assay
Kit size
Total assay time

	ELISA	CLIA
Test performance	Manual or automated	Automated
Random access	Not available	Available
Number of samples	Small or large	Small or large
Throughput	Less or high	High
Operator variability	present	absent
Turn Around time	3 to 4 hours	45 mins to 1 hour
Kits available	Open systems	Closed systems
Variability of results: kit	Present	Absent
Cost	Low	High

CLIA as a screening tool

- Available on an automated platform
- Inherent advantages of automation

✓ reliable
✓ technically precise
✓ increased sensitivity
✓ improved specificity & PPV
✓ high-speed throughput
✓ multi-analyte analysis on a single platform
✓ low consumption of reagents
✓ better stability of reagents and conjugates

Can be used during emergencies since single test is possible & predonation apheresis donor screening due to short TAT and random access

Tiwari et al J Virol Methods 2020

Conclusion

- ELISA & CLIA are recommended for TTI screening
- Choice of testing strategy depends on many factors
- CLIA is more specific
- Automation brings in advantages adding to blood safety

THANK YOU!

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