4th HEPATITIS C
TECHNICAL ADVISORY
GROUP
TAG Meeting

IMPROVE HCV LABORATORY DIAGNOSTICS

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National Center for disease Control and Public health, Georgia
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2017 TAG recommendations

- Patients should not pay for services related to the Elimination Program
- Scale up all components of testing and linkage to care: testing and treatment should happen at same sites
- Testing and treatment should occur at all hospitals, all prisons, all harm reduction, all OST, and all TB and HIV treatment sites
- Simplify testing process
- Quality assurance of HCV diagnostic

IMPROVE HCV LABORATORY DIAGNOSTICS
Progress and Program Outcomes

- Mixed public-private model for the provision of HCV diagnostic and monitoring tests by the National Testing Algorithm
- HCV antibody screening is free of charge for all
- Since December 1, 2017 the confirmation of active HCV infection among anti-HCV screening positive individuals has been provided at no cost
- Since September 1, 2018 the genotyping of HCV individuals with active infection has been provided at no cost

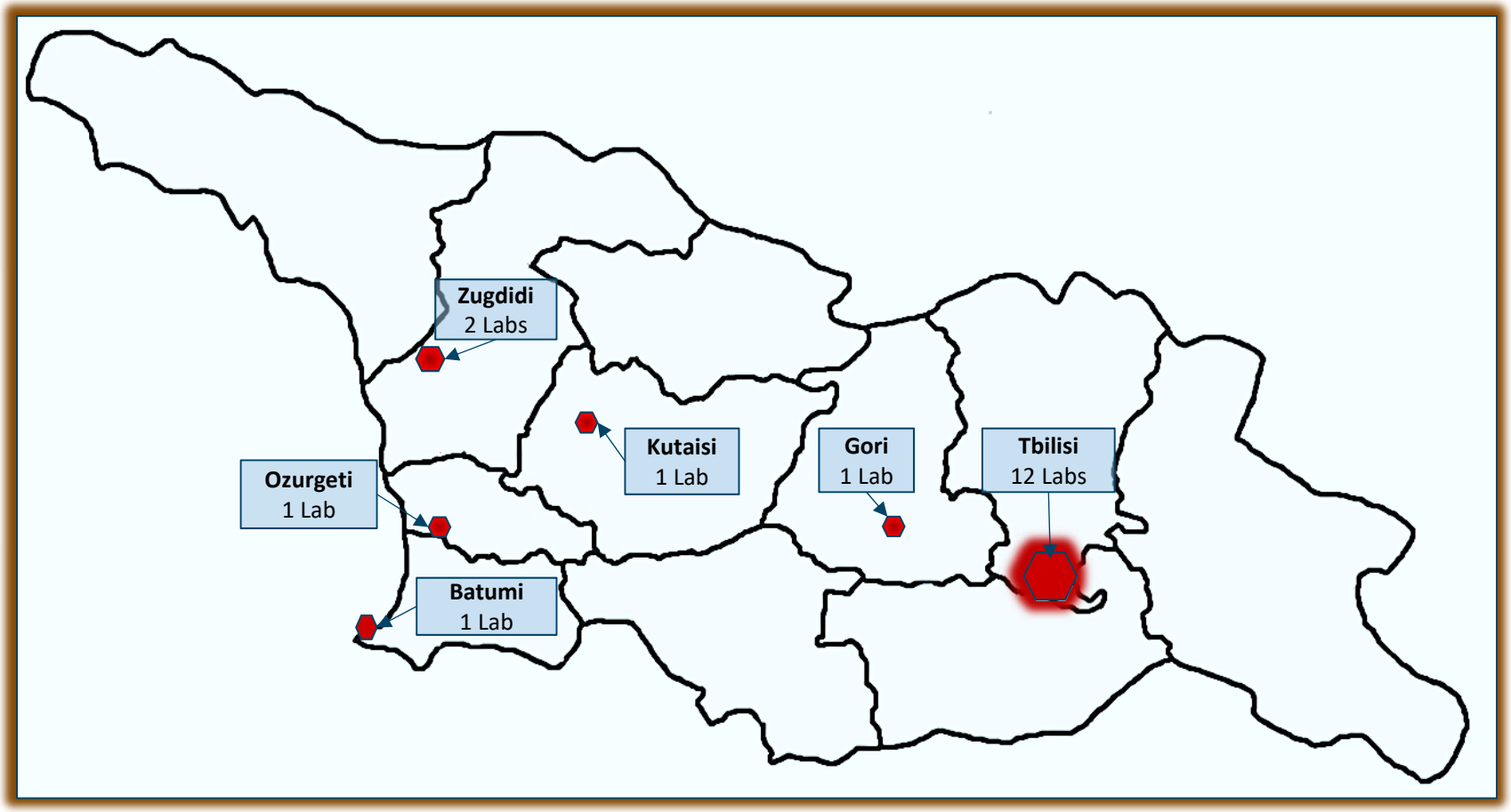
HCV LABORATORY DIAGNOSTICS CAPACITY In GEORGIA

- HCV Elimination Program prompted the initiation of the registration and licensing process for clinical laboratories throughout the country
- More than 500 laboratory service providers have registered in MoLHSA database by December 2017

Region	Clinical laboratories at out-patient units	Clinical laboratories at in-patient units
Tbilisi	183	97
Adjara	15	14
Guria	3	5
Samegrelo-Zemo Svaneti	20	16
Imereti	36	33
Racha-Lechkhumi	-	3
Samtskhe-Javakheti	4	9
Shida-Kartli	8	6
Mtskheta-Mtianeti	-	5
Kvemo Kartli	25	14
Kakheti	11	13

HCV LABORATORY DIAGNOSTICS CAPACITY In GEORGIA

18 (16 private laboratories and 2 public ones) PCR Laboratories within HCV Elimination Program



The Diagnostic Landscape for Hepatitis C infection

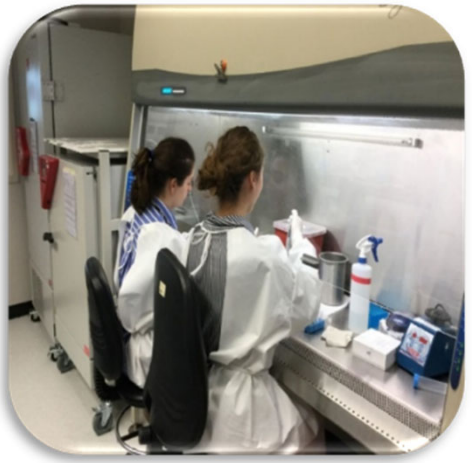
Methods	Facility
Quantitative HCV RNA • All platforms • GeneXpert®	HCV treatment provider sites Harm Reduction centres
Qualitative HCV RNA	HCV treatment provider sites
HCV core Antigen (HCV cAg)	Lugar Center for Public Health Research, NCDC

Identification of active Infection

- Since December 2017, confirmation of anti HCV positive samples from Hospitals using HCV core Ag test has been centralized *(Amendment N532; 7/12/2017)*
  - Lugar Center Serology laboratory is accredited by ANAB according to the clinical laboratory accreditation standard ISO 15189
  - Lugar Center has been receiving about 2000 samples monthly TAT (Turn around Time) is approximately 5 to 10 days, depending on the first result
- From November 2018, confirmation testing of anti HCV positive hospitalized individuals became decentralized
- According the amendment in the Governmental Decree #169 *(Amendment N438; 24.08.2018)* all service providers are allowed to perform first confirmatory tests of anti HCV positive hospitalized individuals using different methods

Improving quality of HCV testing in Georgia through National External Quality Assurance Program

- In 2017 Lugar Center launched the National External Quality Assurance Program
- In March 2017, with technical assistance from the U.S.CDC, the Lugar Center established the first National EQA program for HCV viral load and genotyping
- In 2018 EQA program for HCV qualitative assay was added
- In 2017 Lugar Center enrolled in an international EQA Program with the College of American Pathologists and received serology and molecular panels three times a year.



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EQA Program Results Summary

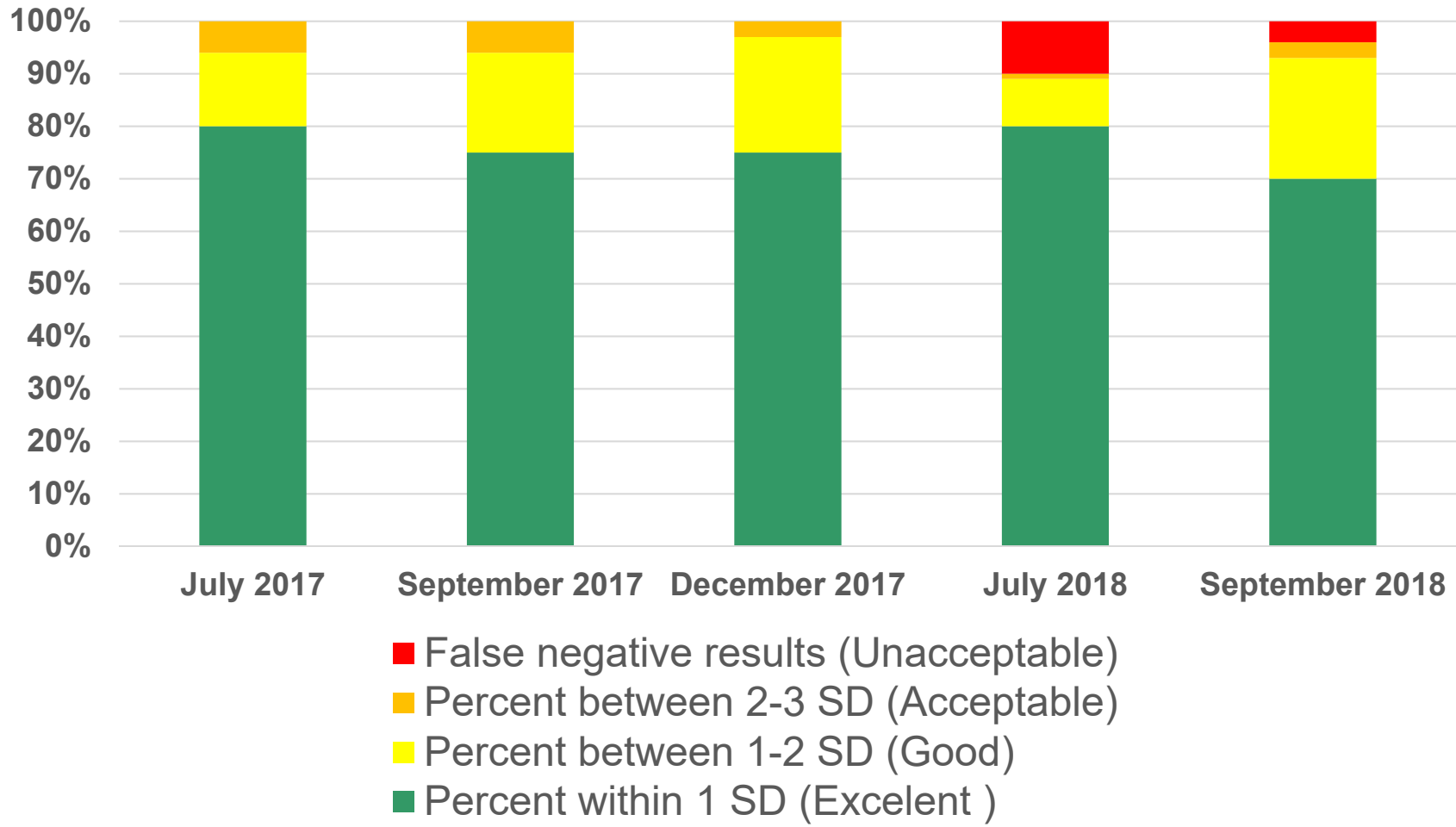
Table 1: QUANTITATIVE VIRAL LOAD

PT Round	# of Labs Reporting	Total # of VL Results	Percent within 1 SD	Percent between 1-2 SD	Percent between 2-3 SD	False negative results
1st July 2017	14/14	70	57 (81,5%)	9 (12,8%)	1 (5,7%)	0
2nd Sept 2017	16/16	80	62 (77,5%)	16 (20%)	2 (2,5%)	0
3th Dec 2017	14*/16	70	54 (77,2%)	14 (20%)	2 (2,8%)	0
4th July 2018	16*/17	80	64 (80,5%)	7 (8,7%)	1 (0,8 %)	8 10%
5th Sept 2018	15*/18	70	50 (71,5%)	15 (21,4%)	2 (2,8%)	3 (4,3%)
Total 2017-2018	Mean	370	287 (78,3)	61 (16,5%)	8 (2,2%)	11 (3%)

*Enrolled laboratories did not participate in December PT round citing insufficient funding/reagents

EQA Program Results Summary - 2017

Figure 1: QUANTITATIVE VIRAL LOAD



EQA Program Results Summary - 2017

Table 2: GENOTYPE

PT Round	# of Labs reporting	# of Labs do not perform test	Genotype	Percent with correct result
1st July 2017	12/14	**2**	3	100%
2nd Sept 2017	14/16	**2**	1b	100%
3rd Dec 2017	12*/16	**4**	1b	100%
4th July 2018	14/17	**3**	1b/2	29%
5th Sept 2018	12*/18	**6**	3	100%

*Enrolled laboratories did not participate in December PT round citing insufficient funding/reagents

EQA Program Results Summary - 2018

Table 3: QUANTITATIVE ASSAY

PT Round	# of Labs reporting	Percent with correct result	Notes
4th July 2018	9*/17	100%	* Only 6 labs use quantitative kits (67%)
5th Sept 2018	12*/18	100%	* Only 6 labs use quantitative kits (50%)

*Enrolled laboratories did not participate in December PT round citing insufficient funding/reagents

Methods, Kits and Equipment

HCV RNA samples preparation methods	HCV RNA Extraction kits
Automatic-Lab Turbo 48 Compact System	Abbott sample preparation kit
Automatic-Cobas Ampli Prep, Roche	Cobas Ampli Prep, HCV test kit, Roche v.2.0
Automatic-croBEE NA16 NA Extr System, GeneProof	Lab Turbo DNA/RNA Mini Kit 48, Taigen Bioscience Corporation
Automatic-NucliSens easy Mag, BioMerieux	RT-GEPATOGEN-C Quant RNA Extraction Kit, DNA Technology
Automatic-Genotraxtract 12, Hain Lifescience	Bosphore Viral RNA Extraction Spin Kit
Semiautomatic- BeadRetriever system	QIAamp Viral RNA Mini Kit, Qiagen GmbH
Manual	Magrev viral DNA/RNA extraction kit, Anatolia Geneworks
	croBEE 201A Nucleic Acid Extraction Kit, GeneProof
	Viral RNA Isolation kit, Human Diagnostic
	NucliSens easy MAG, BioMerieux

Methods, Kits and Equipment

PCR Equipment	HCV RNA VL kits	HCV Qualitative kits	Genotyping kits
Abbott m2000rt	Abbott RealTime HCV kit	HCV Real Time TmQual (Sacache) ref# TVI-100 FRT	**Abbott** RealTime HCV genotype II kit
COBAS Taqman 48 ROCHE	Cobas TaqMan HCV Quantitative Test V2.0, Roche	Bosphore HCV Detection kit V1, Anatolia Geneworks	**Siemens** Versant HCV Genotype 2.0 LIPA
Applied-Biosystems Quant Studio Dx	RoboGene® HCV RNA Quantification Kit 3.0 Germany	HCV RT. Qual. Sacace Biotechnologies	**Sacace Biotechnologies** RTA HCV Genotyping qRCR kit
COBAS 6800 ROCHE	HCV Real TM Quant Dx V1, Sacace Biotechnologies	RT-GEPATOGEN-C Quant PCR Amplif Kit, DNA Technology	**DNA Technology** RT-GEPATOGEN-C Genotype RNA Ampli Kit
Applied-Biosystems Quant Studio 5 RT PCR	Bosphore HCV Quantitation Kit, Anatolia Geneworks	RTA HCV Qualitative Real Time PCR Kit	**Roche** Cobas, HCV Genotyping
Applied-Biosystems 7500 RT PCR	HCV Real TM Quant Dx V1, Sacace Biotechnologies		**Bosphore,** HCV Genotyping kit v1
Thermo fisher Scientific Quant Studio 5 Real-Time PCR System	HCV Real-Time PCR Kit, Human Diagnostic		**NLM, ITALY** HCV Gen-C 2.0
RotorGene 6000 Qiagen	RT-GEPATOGEN-C Quant PCR Amplification Kit, DNA Technology		
DTlite DNA-Technology	Gene Prof Hepatitic ,,C" virus		

EQA Program Results Overview

- As for the qualitative interpretation of HCV RNA test results, no laboratories reported false positive or false negative results, but only (50-67%) use a qualitative kits for detection.
- As for the quantitative of HCV RNA test results, false negative result reported for low VL samples in 2018.
- All laboratories accurately detected common genotype and have partial problem in interpretation of unusual genotype (36%).
- In the comparative analysis of the EQA Program results, a relatively small number of results were between 2 and 3 SD from the mean results.
- Based on those results, the EQA expert and the NCDC EQA staff, assessed the infrastructure, methods and reagents used, and operation processes.
- In most cases, problems included improper use of quantitative PCR calibrators, not following manufacturers' recommendations for PCR platform-reagent combinations;
- Furthermore, the Bosphore Hepatitis kit for HCV genotyping was unable to detect HCV genotype 2 routinely. However, this limitation did not affect the laboratories' performance on the genotype PT challenge.

Conclusions and Recommendations

- Significant diversity of the methods, reagents and equipment
  - Most of tests are CE marked
  - FDA-approved automated tests are most accurate
- Most of the labs modify test methods without validation
  - All the labs should follow the manufacturers recommended protocols, conduct and document test validations
- Limit quantitative VL testing to the labs with outside 2SD
  - Maintain testing by same lab for the duration of DAA therapy in labs with VL variation $\geq$1SD and $\leq$2SD
  - conduct internal test verification for assay performance characteristics: clinical sensitivity and specificity, accuracy, reproducibility (precision), and limit of detection (LOD)
- Laboratory with unacceptable result outside <-2SD or >+2SD, false negative results need to assess nonconformities and elaborate plan for corrective actions

Future Directions

- Establish HCV serology EQA
  - Blood banks
  - Rapid screening tests
- Maintain schedule of 3 PT panels per year for all registered laboratories
- Expand the pool of PT specimens to include additional samples with low VL for qualitative & quantitative assay, genotypes
- Organizing site visit of participant laboratories with non compliant result to assessing problems and developing recommendation for improvement
- Expand the number of laboratories participating in the National HCV EQAP

Monitoring and Evaluation: Laboratory Diagnostics, 2017

4.1 Improve laboratory detection of HCV infection

Indicator name	Measurement	Data source	Value/Result	Remarks
1. Proportion of HCV confirmatory testing sites (laboratories and point of care diagnostic sites) enrolled in the national hepatitis C EQA program	**Numerator** Number of laboratories performing HCV confirmatory testing that are enrolled in national/international hepatitis C EQA program **(N=16)** **Denominator** Total number of laboratories performing HCV confirmatory testing in Georgia **(N=16*)**	NCDC Lugar Center, MoLHSA	**100%**	*Denominator includes the reference lab- Lugar Center
2. Proportion of HCV confirmatory testing sites that participated on 3 EQA challenges per year	**Numerator** Number of laboratories performing HCV confirmatory testing that participated on 3 National/international EQA challenges per year **(N=12)** **Denominator** Total number of laboratories performing HCV confirmatory testing enrolled in hepatitis C EQA program **(N=16)**	NCDC Lugar Center EQA Program	**75%**	

Monitoring and Evaluation: Laboratory Diagnostics, 2017

4.1 Improve laboratory detection of HCV infection

Indicator name	Measurement	Data source	Value/Result	Remarks
3. Quality Management System standards for certification are defined, approved, and published		Published QMS standards, MoLHSA	In process	
4. Proportion of labs providing HCV lab services certified according to national laboratory quality management system (QMS) standards	**Numerator** Number of laboratories performing hepatitis C laboratory services that are certified according to national QMS standards **Denominator** Total number of laboratories performing hepatitis C laboratory services	MoLHSA	Data not available	

Acknowledgements

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