ESGMD

ESCMID STUDY GROUP FOR GENOMIC AND MOLECULAR DIAGNOSTICS

European Society of Clinical Microbiology and Infectious Diseases

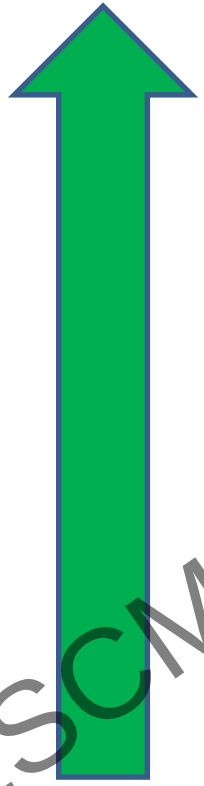
Quality Control for Molecular Diagnostics

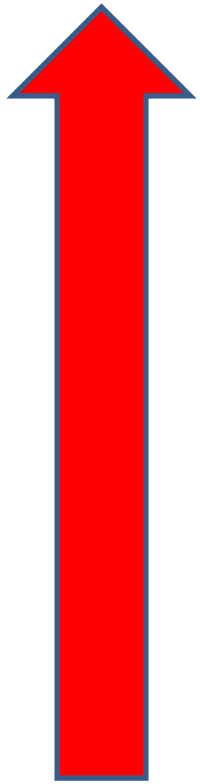
Prof. Jacob Moran-Gilad, MD MPH

on behalf of ESGMD

Framework

- In house diagnostics – home brew assays
- Commercial diagnostics – ‘kitified’ assays
- Commercial diagnostics – ‘blackbox’ assays

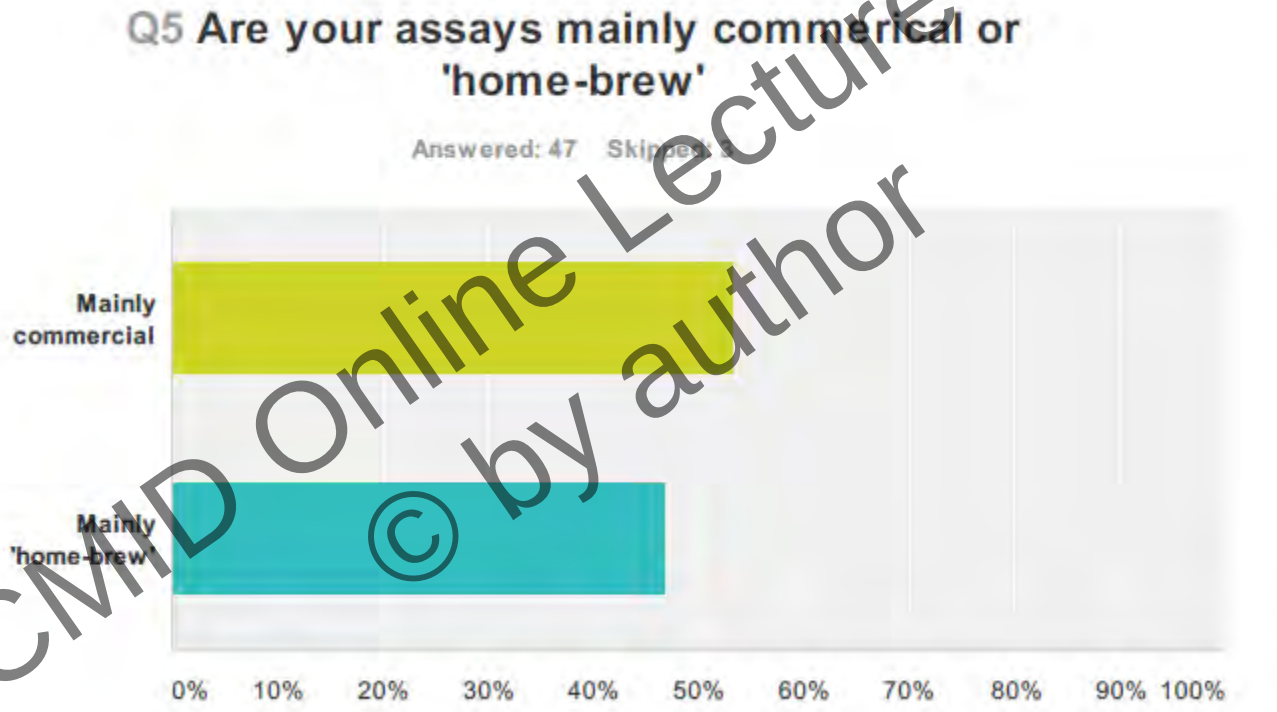




Flexibility

Risk

Framework



Current issues & gaps

- Clinical indications for testing
- Choosing the right sample for the right test
- Meaningful cut-off values
- Multiplex to the max
- Minimal requirements – black box mentality
- Harmonisation of guidelines
- Application of guidelines

Diagnostic hierarchy

- Detection > Quantitation > Characterisation
- POCT > clinical > regional > reference

Process analysis

- (Infra-structure)
- Development plan
- Setting up and Optimisation
- Analytical validation
- Clinical Validation
- Performance & QC
- Roll out & verification
- Surveillance

When can it go wrong?

- Non-indicated/non-validated usage
- Inadequate verification during roll out
- Rare diseases
- Leap from secondary to primary testing
- Assay tweaking
- Inadequate controls
- Shelf-life issues
- Ad hoc testing
- Suboptimal routine QA/QC

Validation req.

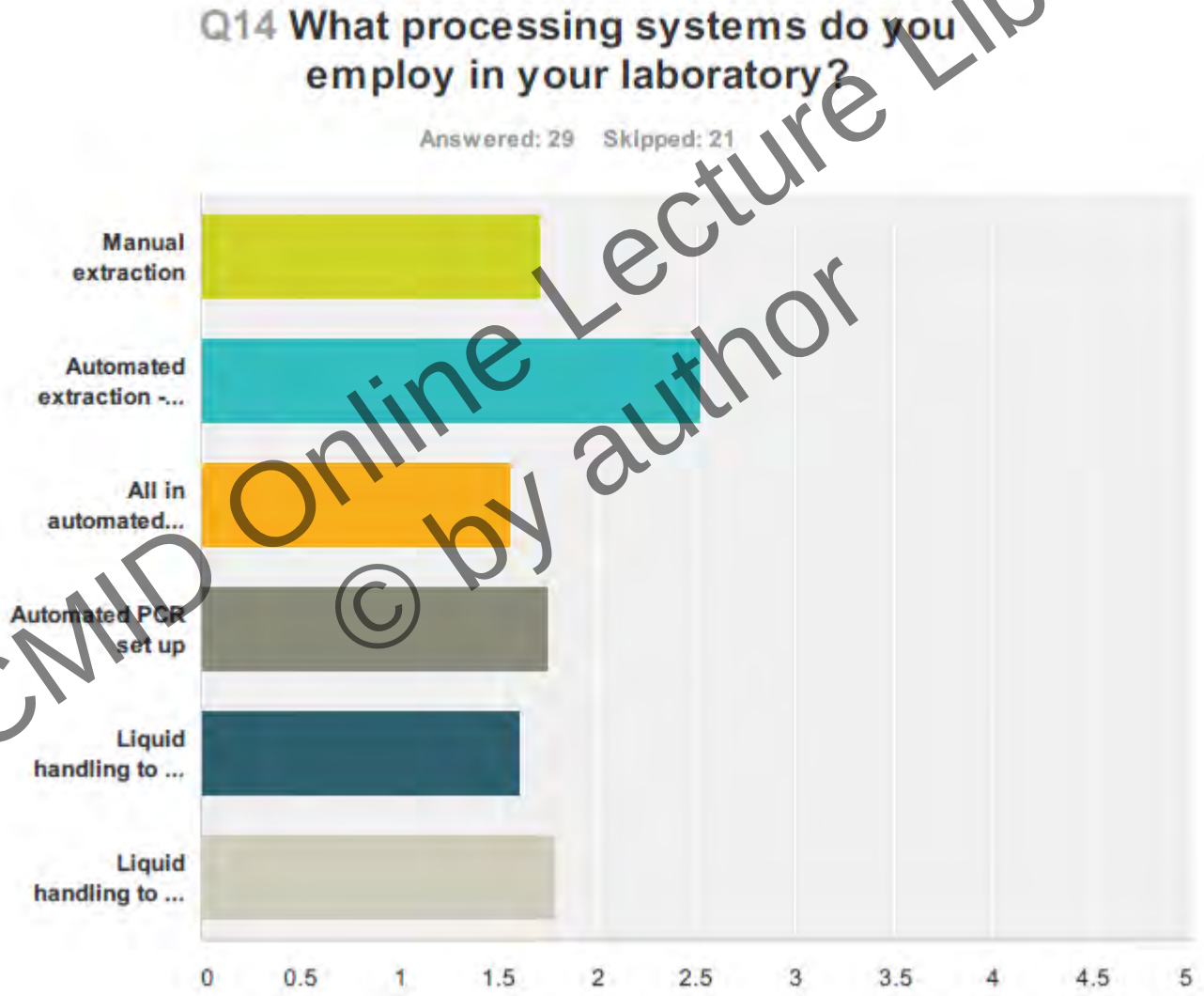
Parameter	Design
Analytical sensitivity	Four different assay runs with at least three replicates per dilution of the sample.
Diagnostic sensitivity	Testing of samples (that have been tested using the 'gold-standard' or appropriate alternative assay) from cases with the defined clinical profile(s). The minimum numbers of samples to be tested (to give a required level of reliability of the sensitivity measurement) will depend on the prevalence of disease and can be calculated from the minimum sensitivity levels and the 95% CI shown in table I.
Analytical specificity	Testing of DNA extracted from as many variants as possible of the target organism, genetically related organisms and organisms likely to be found in positive and negative cases (>100) with the defined clinical profile(s).
Diagnostic specificity	Testing of >50 samples that were positive and >50 samples that were negative using the gold-standard assay.
Efficiency (quantitative assays)	Test 10 fold dilutions of a positive sample or control in triplicate. Dilution range to give C_qs from <12 to >35 cycles.
Linearity (quantitative assays)	Test 10 fold dilutions of a positive sample or control in triplicate. Dilution range to give C_qs from <12 to >35 cycles.
Measurement range	Test 10 fold dilutions of a positive sample or control in triplicate. Range to extend from lowest practical dilution to ten fold beyond highest dilution giving a positive result.
Precision (quantitative assays)	Three samples (high medium and low positive) assayed at least four times or more in one run and over at least four different runs on different days.
Reproducibility (quantitative assays)	Three samples (high medium and low positive) assayed at least four times or more in one run and in at least four different runs on different days. These to be run in different laboratories or using different reagent batches or different instruments.
Analytical accuracy (quantitative assays)	Three analytical standards (high medium and low positive) assayed at least four times or more in one run and over at least four different runs on different days.
Clinical accuracy (quantitative assays)	Three clinical standards (high medium and low positive) assayed at least four times or more in one run and over at least four different runs on different days.
Reference intervals	Testing of >100 samples (that have been tested using the gold-standard assay) from cases with the defined clinical profile(s).
Clinical validation	Analysis of samples from cases with the defined clinical profile(s) with follow-up. This is on-going audit of assay performance.
Shelf-life	Samples from three batches stored at the designed storage temperature. Aliquots used to assay three samples (high medium and low positive) at least four times or more in one run and in at least two different runs on different days.

Revalidation req.

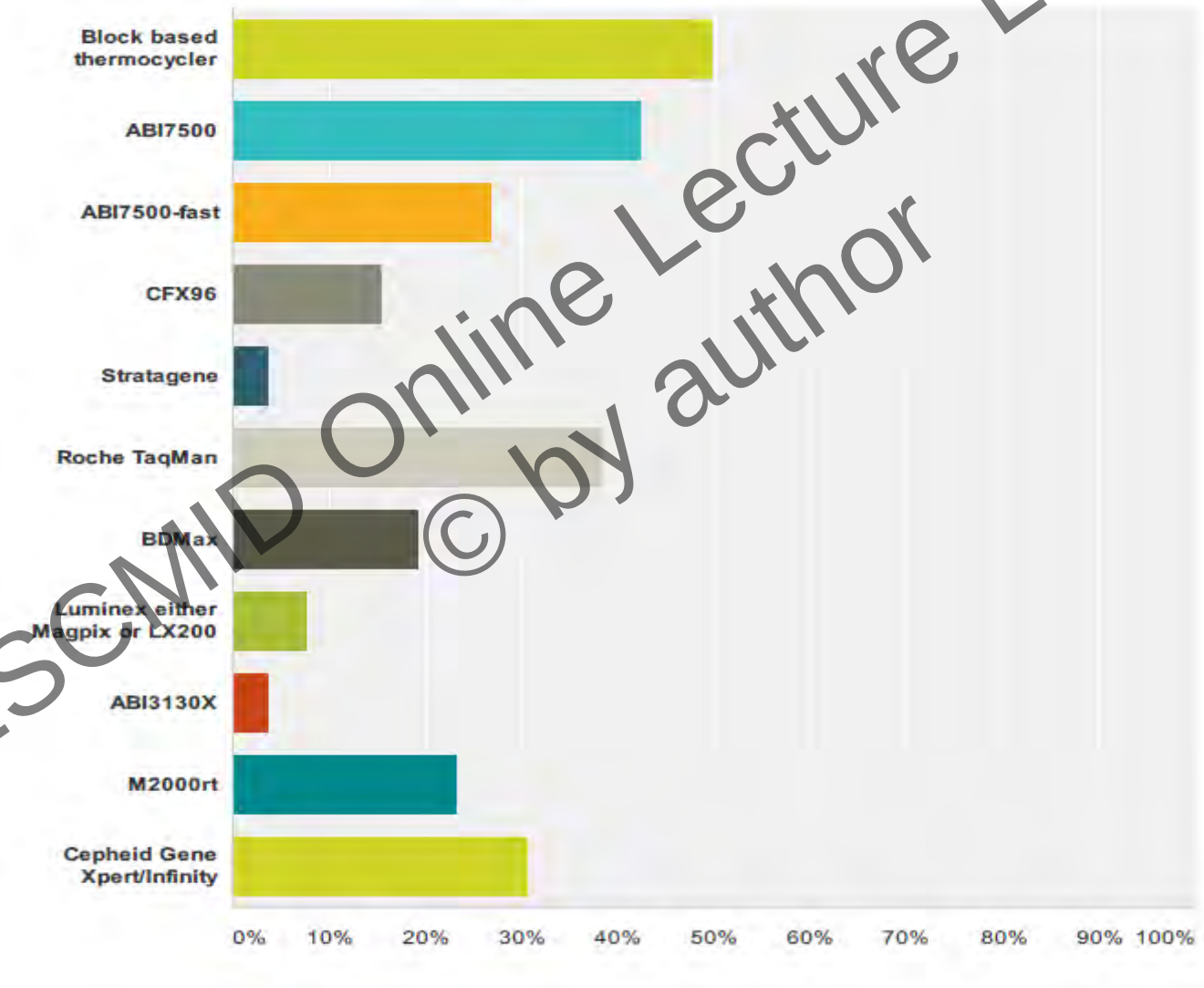
Annex G - A guide to the level of revalidation considered acceptable for a range of changes to the protocol

	Protocol change					
Revalidation activity	primers/ probes	reaction mix components (ex – primers/ probes)	extraction method	control material	instrument or conditions	analysis
Analytical sensitivity	yes	yes	yes	no	yes	yes
Diagnostic sensitivity	yes	*	yes	no	*	*
Analytical specificity	yes	*	limited$	limited	limited	limited
Diagnostic specificity	yes	*	*	no	*	*
Efficiency (quantitative assays)	yes	yes	no	no	yes	yes
Linearity (quantitative assays)	yes	*	yes	no	*	*
Measurement range	yes	*	*	no	*	*
Precision (quantitative assays)	yes	*	yes	no	*	*
Reproducibility (quantitative assays)	yes	*	yes	no	*	*
Analytical accuracy (quantitative assays)	yes	*	yes	yes	*	*
Clinical accuracy (quantitative assays)	yes	*	yes	no	*	*
Reference intervals	yes	*	*	no	*	*
Clinical validation	yes	*	*	no	*	*
Shelf-life	partial&	partial	no	partial	no	no

Technologies



Technologies



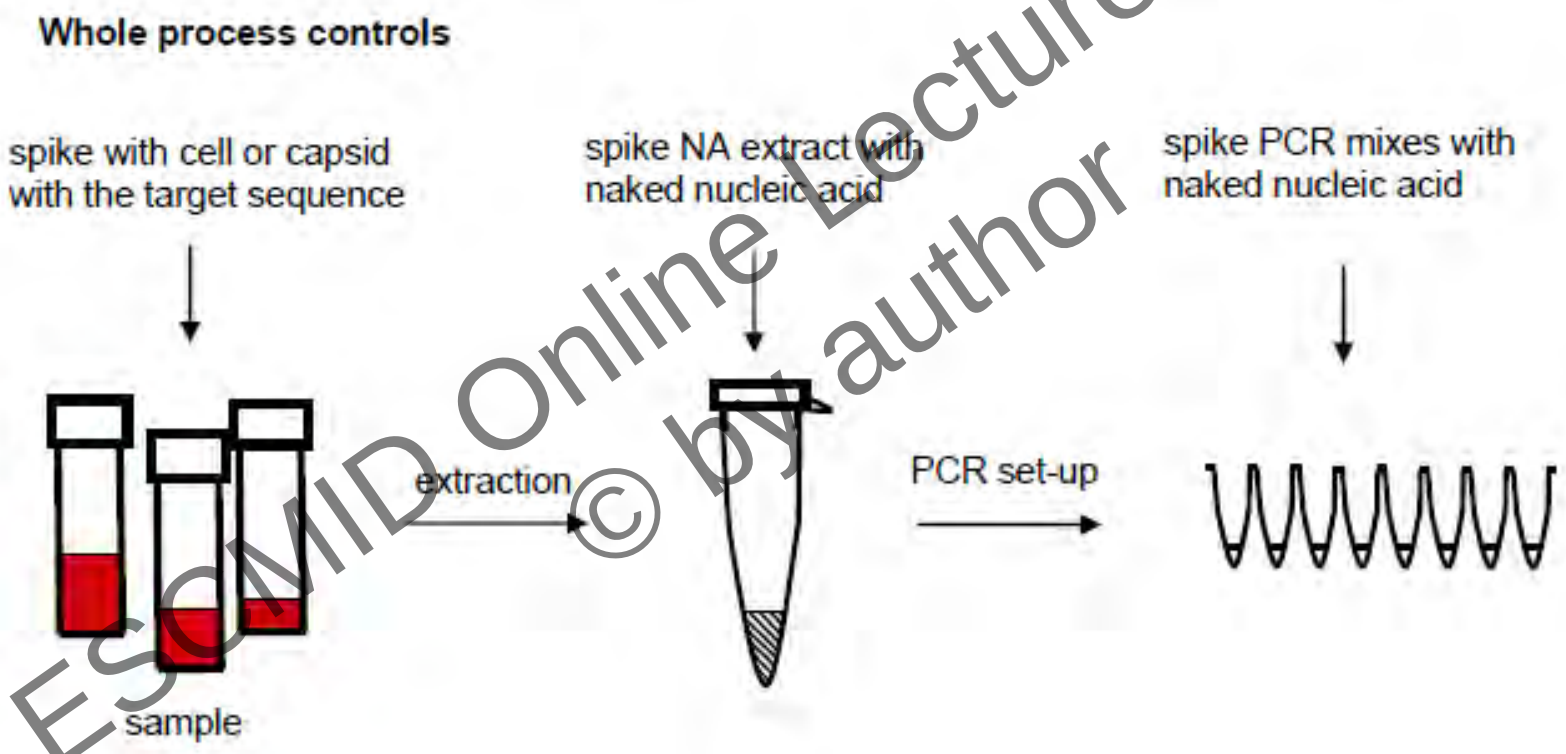
The greatest concern

- A false negative critical qPCR resulting from:
  - Faulty extraction
  - Amplification inhibition
  - Poor diagnostic accuracy
  - Suboptimal routine QA/QC

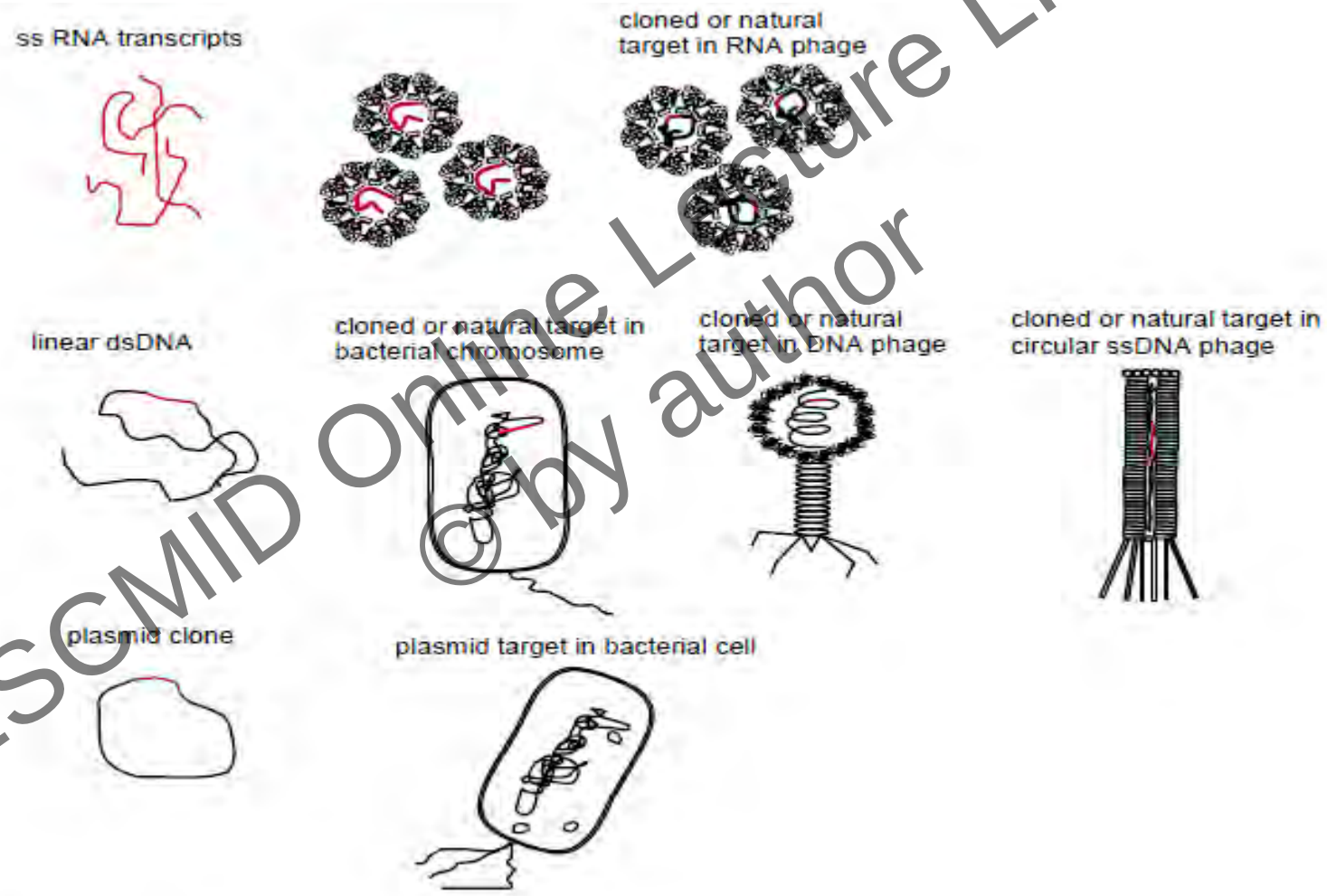
PCR controls

- Batch vs. Run vs. Sample
- External vs. Internal
- Partial vs. Whole process
- Qualitative vs. Quantitative

PCR controls



PCR controls



Target sequence is shown in red and carrier nucleic acid in black.

PCR controls

Extraction control	Inhibition control	Degree of assurance
Control (phage coated RNA and/or DNA as appropriate) spiked into each sample	Extracted positive nucleic detected in same tube	Good
Human DNA indicative of good sample	Extract tested for intrinsic human template in the same tube	Good
Generic target (typing or gene variant assay)	Generic target detected in same tube	Good
Positive control cells extracted in separate tube	Positive target spiked into each sample detected in same tube	Good
No extraction control	Positive target spiked into each sample detected in same tube	Acceptable
Control DNA (e.g. plasmid) spiked into each sample	Extract tested for control template in a parallel tube	Acceptable
Human DNA indicative of good sample	Extract tested for intrinsic human template in a parallel tube	Acceptable
Extraction of nucleic acid tested by a parallel assay	Inhibition tested by a parallel assay	Low
Batch extraction control	Extract tested for spiked low copy template in a parallel tube	Low
No extraction control	Extract tested for spiked control template in a parallel tube	Low

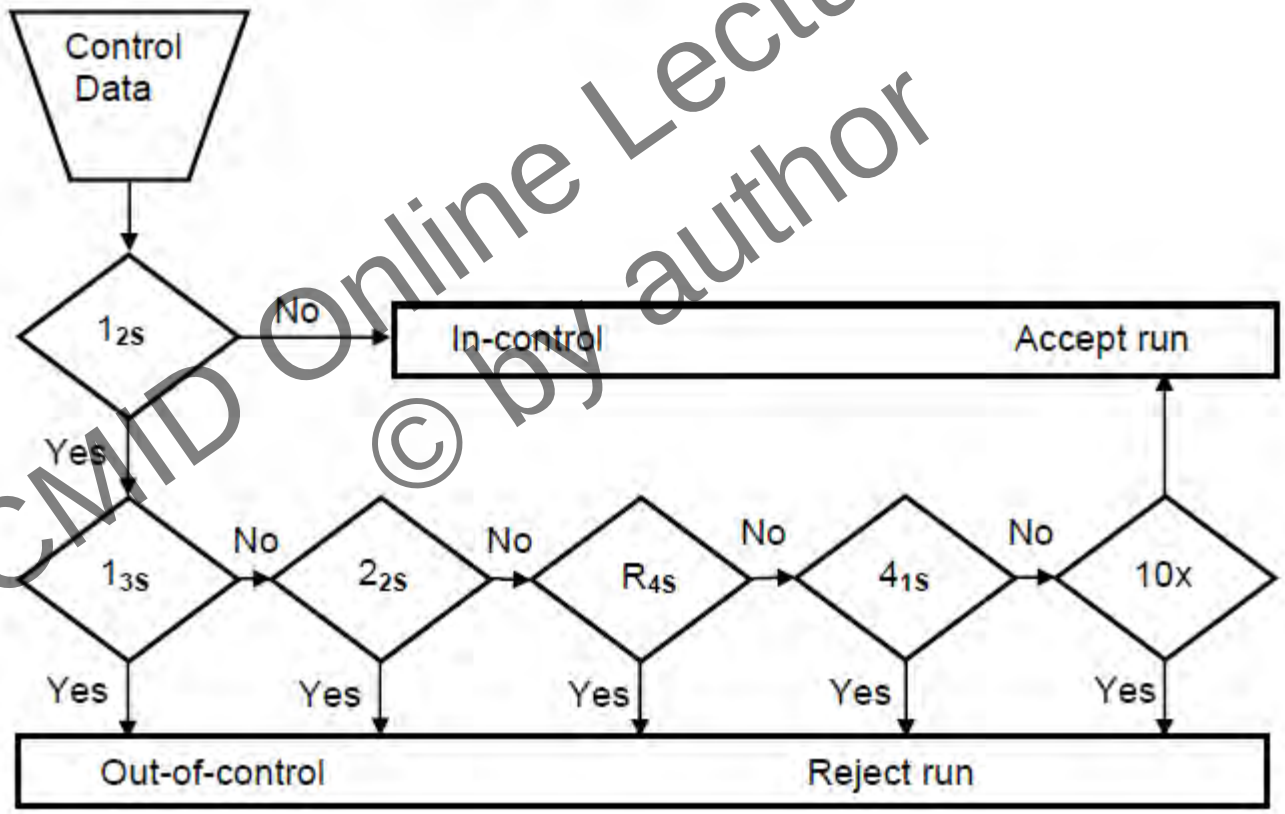
	Internal controls		External controls

PCR controls

Answer Choices	Responses	
Positive control which is extracted as well as PCR	57.69%	15
Positive control - PCR only	57.69%	15
Negative Control	100.00%	26
Internal control - amplification control only	46.15%	12
Internal control - extraction and co-amplification	61.54%	16
Internal control with target specific primers	26.92%	7
Internal control which is generic across many assays	30.77%	8
Monitoring of run control to conform to Westgard Rules	19.23%	5
Use of international units where these are available	38.46%	10

Routine QA/QC

Figure. A typical use of Westgard rules



Vision of WG 4 - Proficiency Testing

That all laboratories globally conducting NGS on bacteria and virus to the highest degree of quality in terms of detection of relevant genes, point mutations and phylogeny

Mission of WG 4 - Proficiency Testing

To provide a formal mechanism for inter-laboratory test performance to ensure harmonization and standardization in whole genome sequencing and data analysis, with the aim to produce comparable data

Goal of WG 4 - Proficiency Testing

- To organise a proficiency test for WG participants
- To offer this test to GMI members working with both bacteria and virus

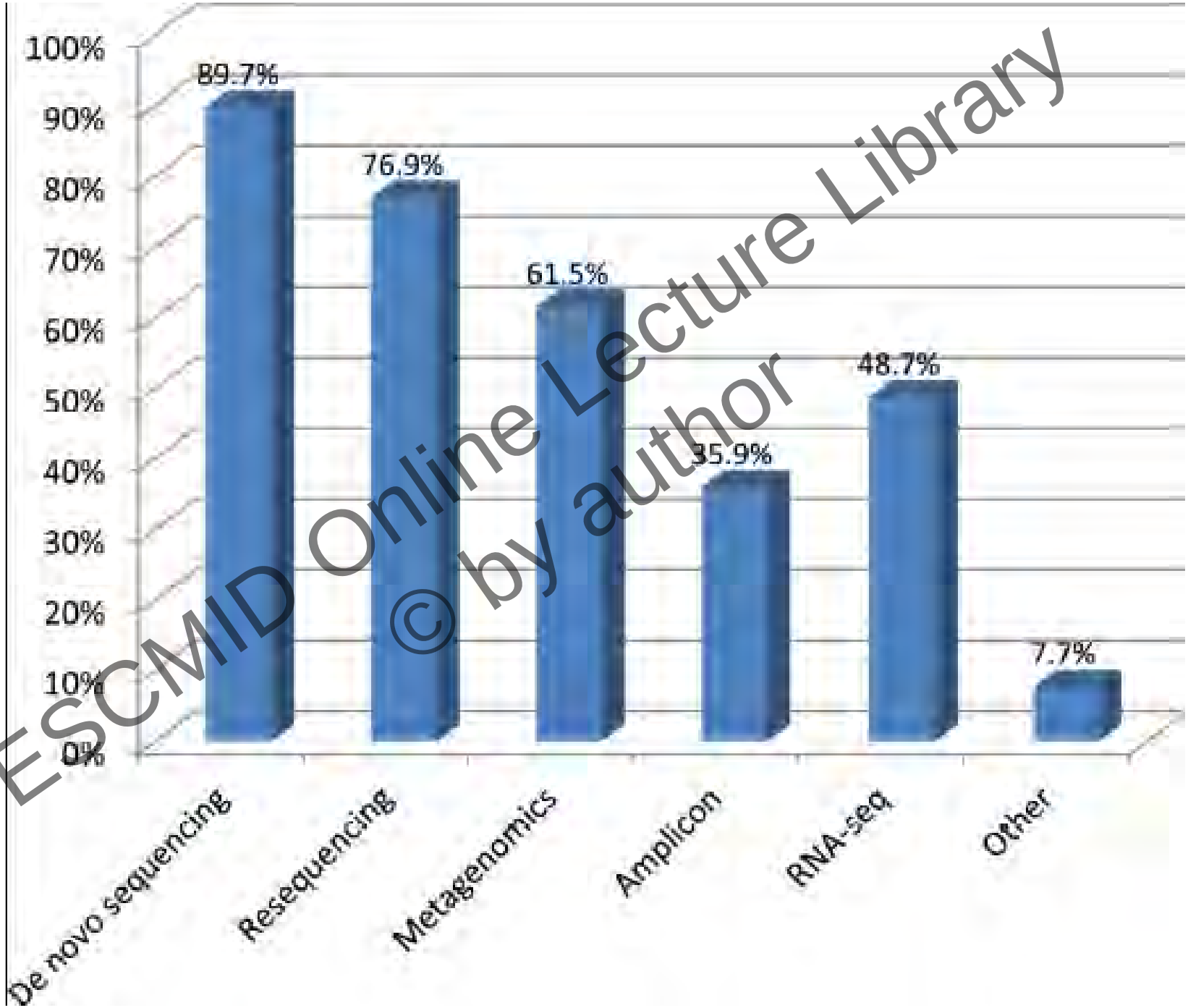
Moran-Gilad *et al. BMC Infectious Diseases* (2015) 15:174
DOI 10.1186/s12879-015-0902-3

BMC
Infectious Diseases

CORRESPONDENCE Open Access

Proficiency testing for bacterial whole genome sequencing: an end-user survey of current capabilities, requirements and priorities

Jacob Moran-Gilad[1,2], Vitali Sintchenko[3,4], Susanne Karlsmose Pedersen[5], William J Wolfgang[6], James Pettengill[7], Errol Strain[7], Rene S Hendriksen[5*] and on behalf of the Global Microbial Identifier initiative's Working Group 4 (GMI-WG4)



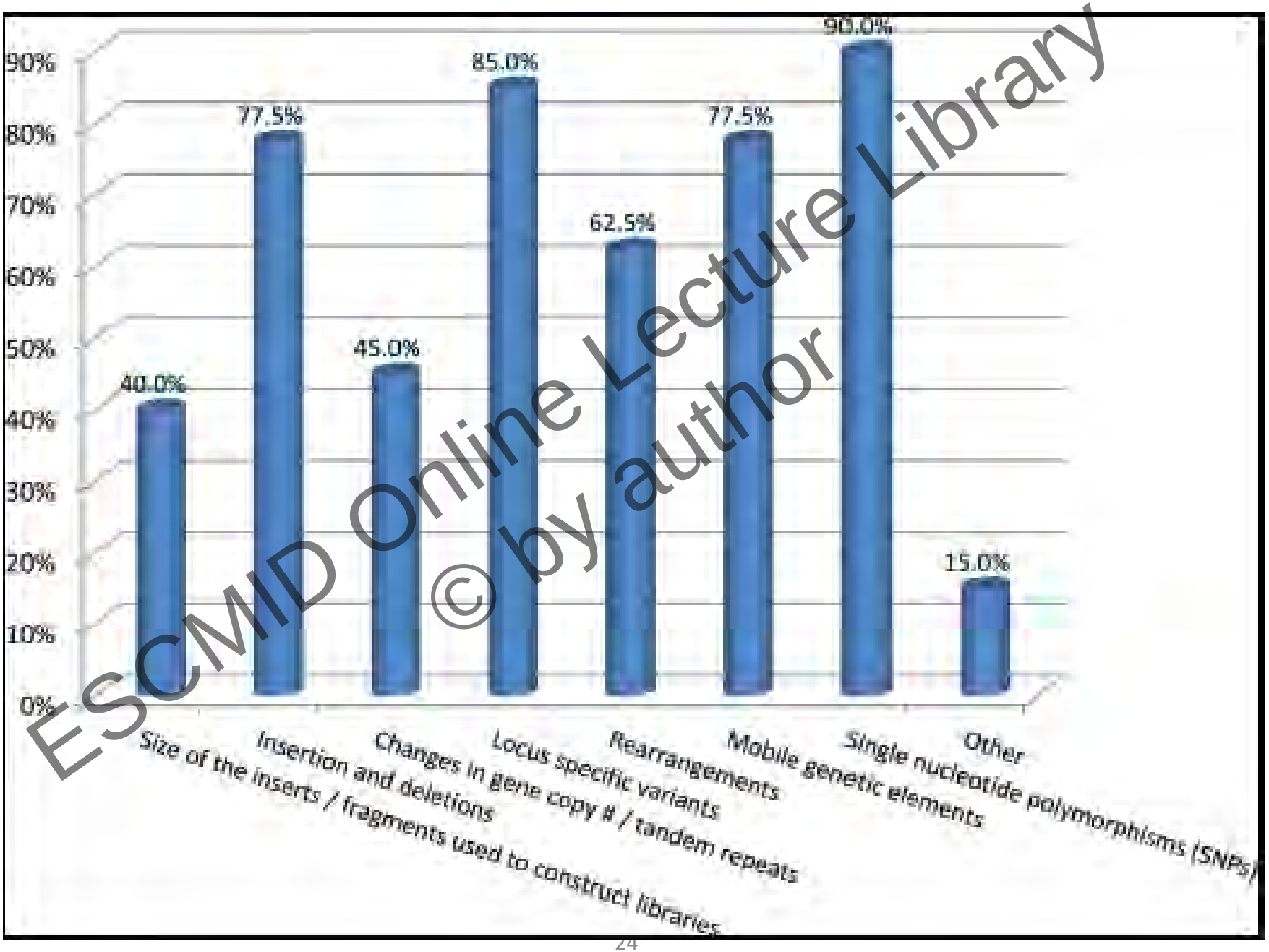


Figure S3. Distribution of NGS access across technologies

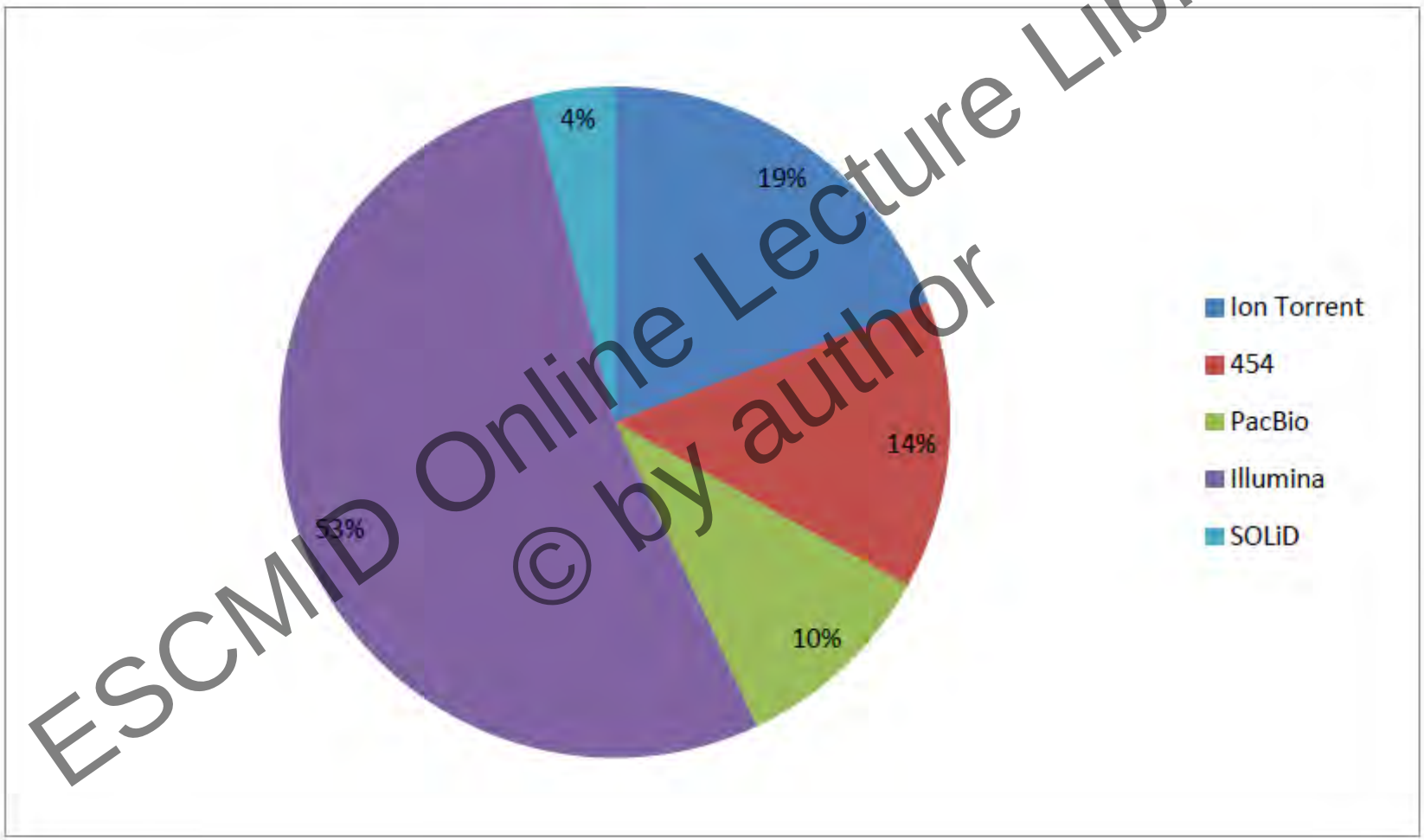


Figure S2a. NGS capability of participating institutions

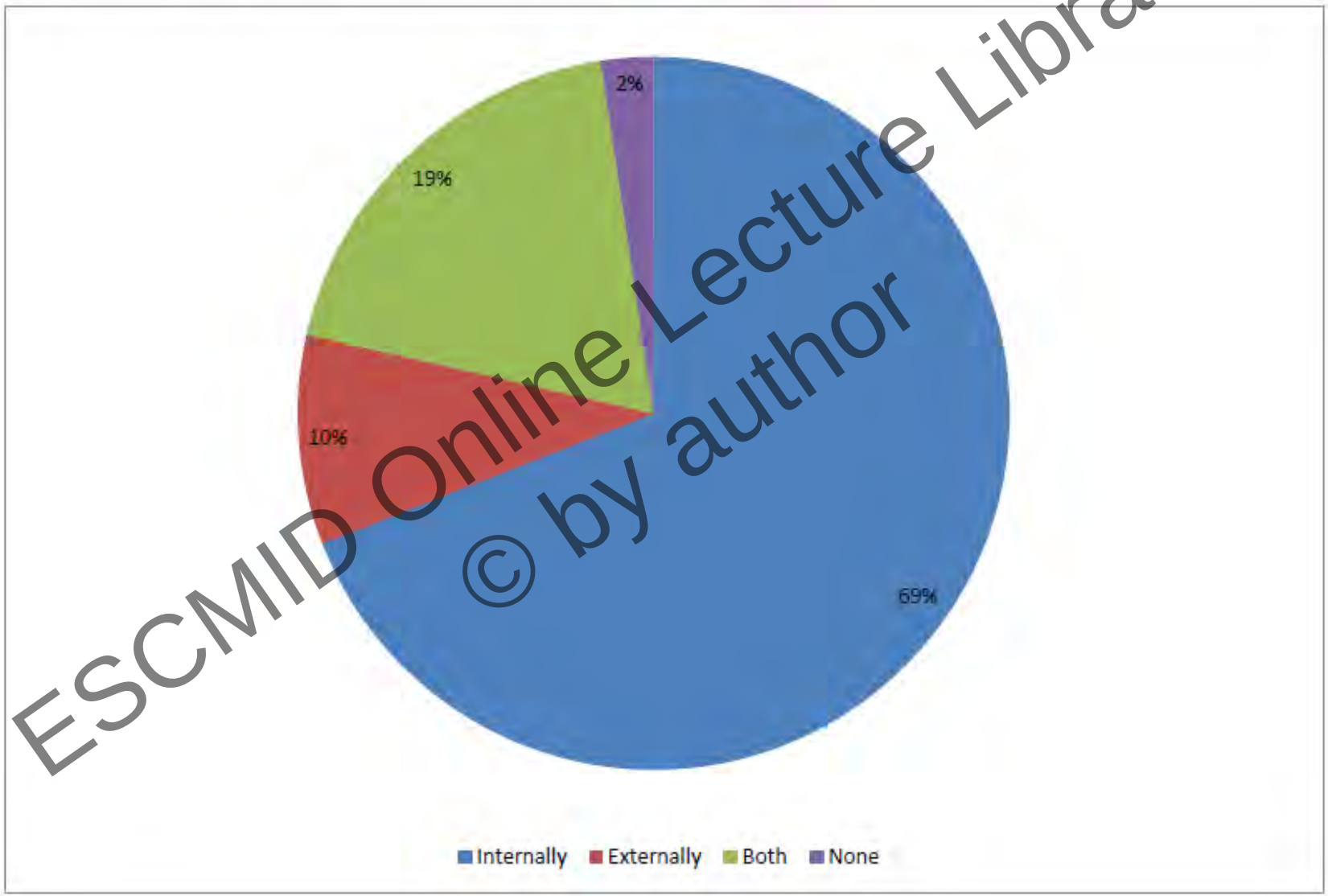
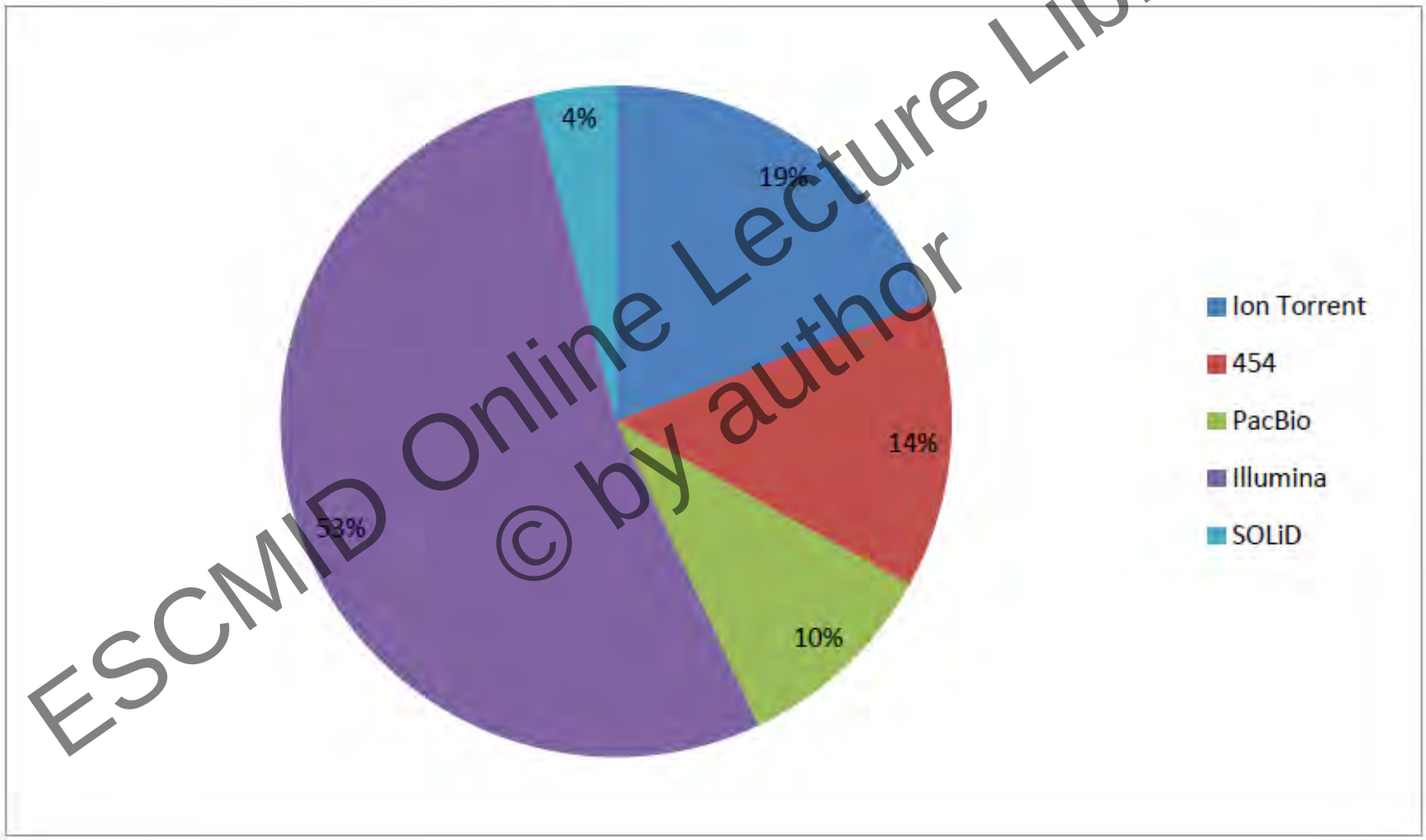
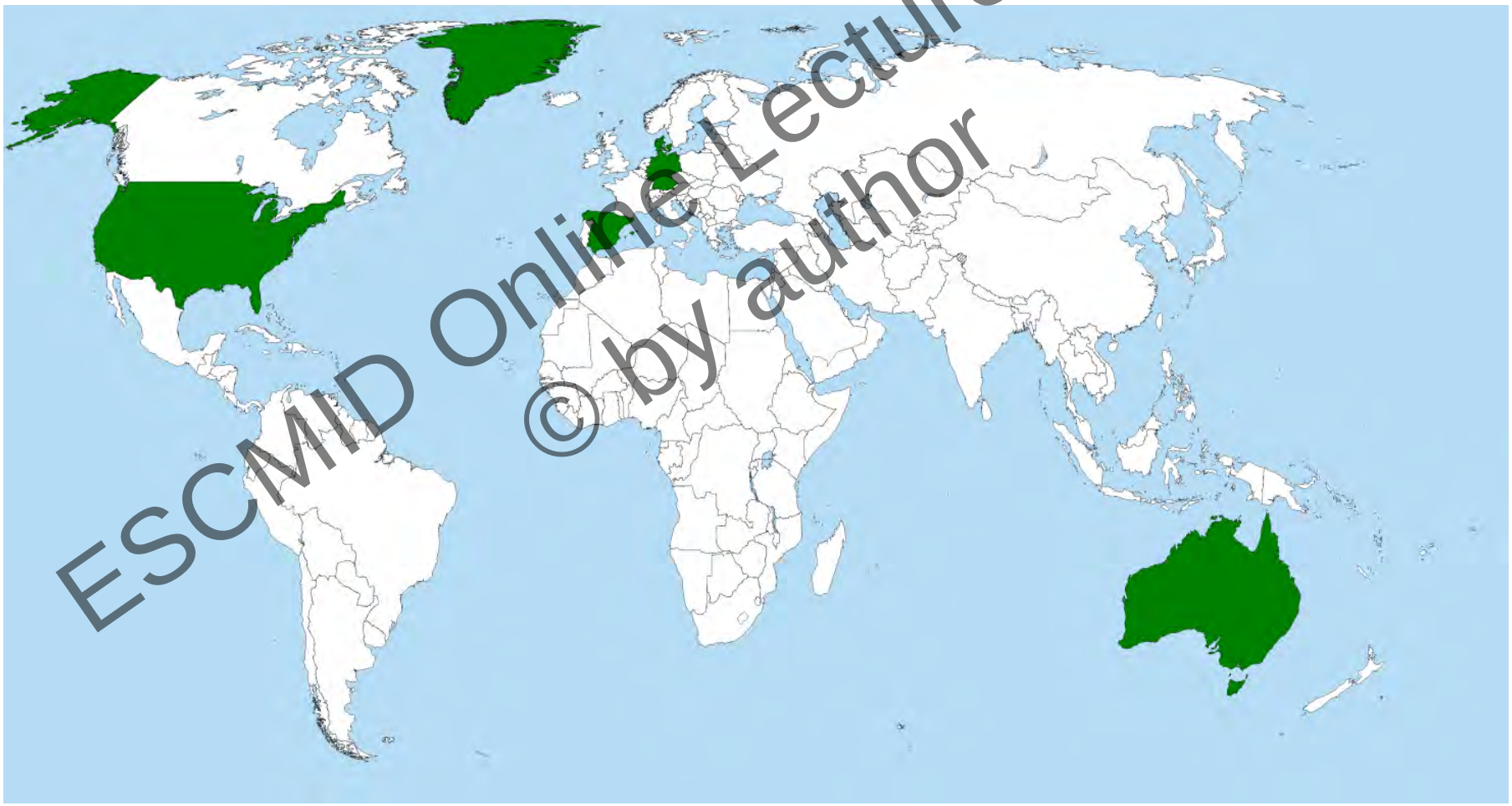


Figure S3. Distribution of NGS access across technologies



GMI WG 4 pilot proficiency test – Deliverable #2

Countries participating in the GMI pilot PT 2014



GMI WG 4 pilot proficiency test – Deliverable #2

The pilot PT consist of three components

- 1a) DNA extraction, purification, library-preparation, and whole-genome-sequencing of six bacterial cultures;
  - two *Salmonella* strains
  - two *Escherichia coli* strains (only one was included)
  - two *Staphylococcus aureus* strains

  Upload reads to an ftp-site

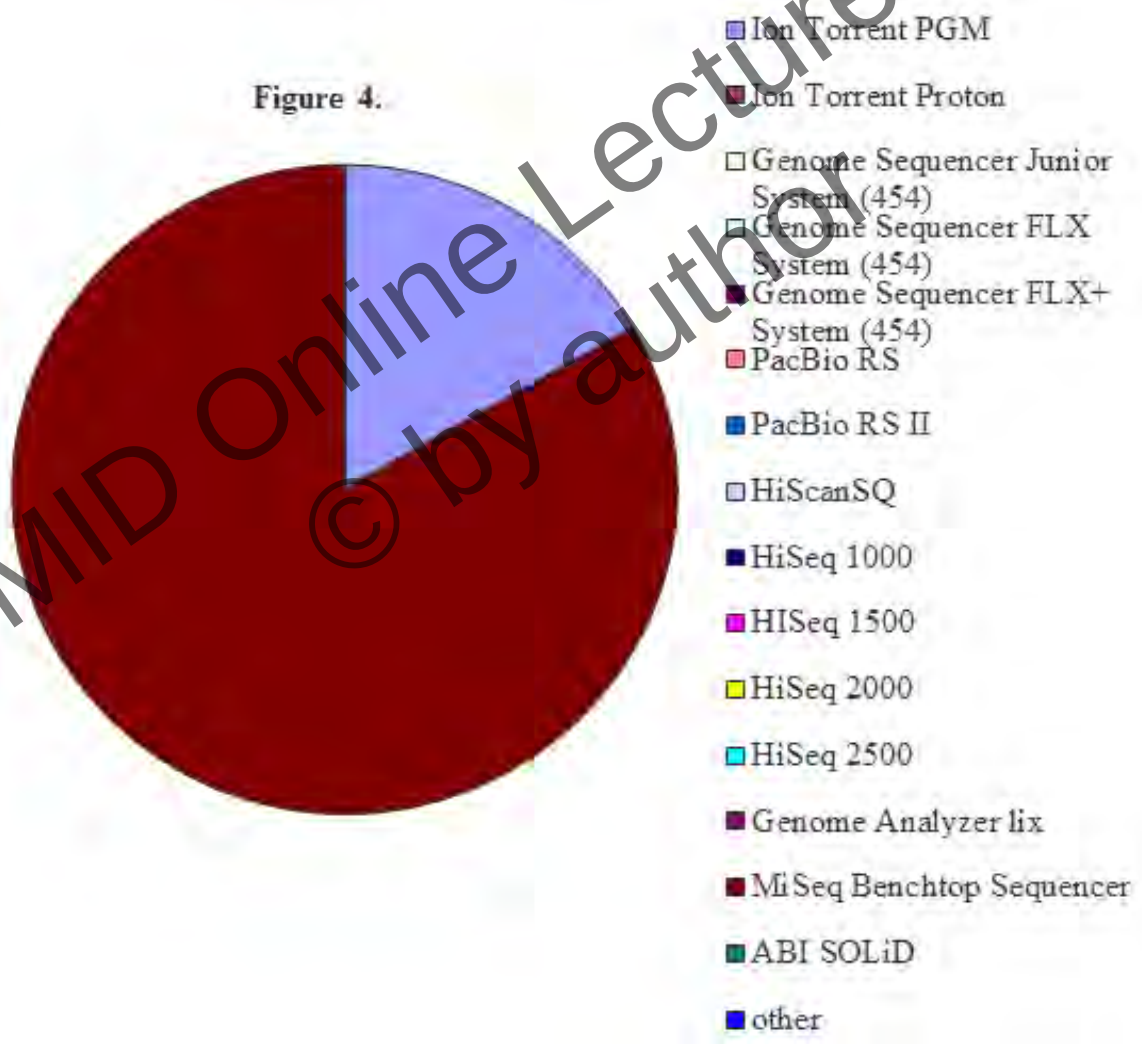
  Optionally, identify MLST and resistance genes present in the strains

- 1b) Whole-genome-sequencing of pre-prepared DNA of the same six bacterial strains mentioned in component 1a for comparison of DNA and library prep

- 2) Phylogenetic/clustering analysis of three datasets each including fastq data from app. 20 genomes of *S.* Typhimurium, *E. coli* and *S. aureus*

GMI WG 4 pilot proficiency test – Deliverable #2

Applied sequencing platform used in the proficiency test



GMI WG 4 pilot proficiency test – Deliverable #2

Overview of participants' use of platforms and tools

Participant	1	2	3	4	9
Platform	Miseq	Miseq	IonTorrent	Miseq	Miseq
Read length	150	300	200	300	301
Read type	Paired-end	Paired-end	Single-end	Paired-end	Paired-end
Assembler*	Velvet	-	CLC Genomics Workbench 7	https://cge.cbs.dtu.dk/services/Assembler/	Velvet, https://www.ebi.ac.uk/~zerbino/velvet/, open access
Applied tools, MLST	Inhouse script	http://cge.cbs.dtu.dk/services/MLST/	MLST Databases of UoW (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/); (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) and Staphylococcus aureus MLST database (http://saureus.mlst.net/)	http://cge.cbs.dtu.dk/services/MLST	SRST2 with MLST databases from http://pubmlst.org, http://katholt.github.io/srst2/, open access
Applied tools, resistance genes	Inhouse script, ResFinder	http://cge.cbs.dtu.dk/services/ResFinder/	In-house custom pseudomolecules	http://cge.cbs.dtu.dk/services/ResFinder	SRST2 with ResFinder resistance gene database, http://katholt.github.io/srst2/, open access

GMI WG 4 pilot proficiency test – Deliverable #2

Determined MLST and antimicrobial resistance genes in Salmonella GMI14-002

	BACT										DNA									
Participant	1		2		3		4		9		1		2		3		4		9	
	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool
Expected MLST	ST599																			
Obtained MLST	599	599	599	599	599	599	599	599	Unk.	599	599	599	599	599	599	599	599	599	Unk.	599
MLST as expected	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y
aroC	14	14	14	14	14	14	14	14	599	14	14	14	14	14	14	14	14	14	599	14
dnaN	7	7	7	7	7	7	7	7	14	7	7	7	7	7	7	7	7	7	14	7
hemD	3	3	3	3	3	3	3	3	7	3	3	3	3	3	3	3	3	3	7	3
hisD	12	12	12	12	12	12	12	12	3	12	12	12	12	12	12	12	12	12	3	12
purE	6	6	6	6	6	6	6	6	12	6	6	6	6	6	6	6	6	6	12	6
sucA	19	19	19	19	19	19	19	19	6	19	19	19	19	19	19	19	19	19	6	19
thrA	12	12	12	12	12	12	12	12	19	12	12	12	12	12	12	12	12	12	19	12

GMI WG 4 pilot proficiency test – Deliverable #2

Determined MLST and antimicrobial resistance genes in Salmonella GMI14-002

	BACT										DNA									
Participant	1		2		3		4		9		1		2		3		4		9	
	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool	Own tool	CGE tool
$bla_{CTX-M-15}$	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X
bla_{SHV-12}	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
bla_{TEM-1b}	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
catA2	X*						X		X		X*						X		X	
dfrA18	X*	X		X		X	X	X	X	X	X*	X		X		X	X	X	X	X
dfrA19					X										X					
ere(A)	X*	X		X	X	X	X	X	X	X	X*	X		X	X	X	X	X	X	X
floR	X*	X	X	X		X	X		X	X*	X	X	X		X		X	X		X
pbrA-pbrB					X										X					
qacEdelta1					X										X					
QnrB2					X				X						X				X	
QnrB49	X*	X		X		X	X	X		X	X*	X		X		X	X	X		X
strA	X	X	X	X		X	X	X	X		X	X	X			X	X	X	X	X
strB	X	X		X		X	X	X	X	X	X	X		X		X	X	X	X	X
sul1	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X
sul2	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X
tet(A)	X*	X	X	X	X	X	X	X		X	X*	X	X	X	X	X	X	X		X
tet(D)	X	X		X		X	X	X	X	X	X	X		X		X	X	X	X	X
aac(3)-IIb							X										X			
aac(6')-IIc	X	X		X		X	X	X	X	X		X		X		X	X	X	X	X
aac(6')-Iy	X*	X	X	X		X	X	X	X	X	X*	X	X	X		X	X	X	X	X
aac3					X										X					
aacA27					X										X					
arr					X										X					

Susanne Karlsmose
Mette Voldby Larsen
Anthony Underwood
Errol Strain
Brian Beck
Nate Olson
Isabel Cuesta de la plaza
Angel Zaballos
Jorge De La Barrera Martinez
Vitali Sintchenko
Bill Wolfgang
Jacob Moran Gilad
Henrik Westh
William Hsiao
Andreas Nitsche …..and the rest of WG 4