SCINOTE

Laboratory automation and data management in diagnostics

qPCR ZIKV detection and quantification

Matjaz Hren, PhD

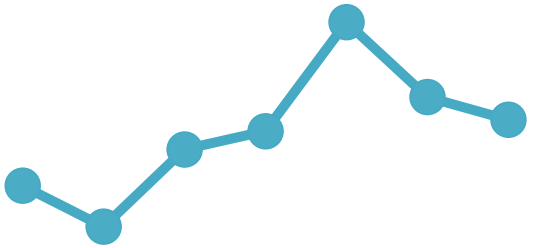
PAIN

3 years

Scientific data output doubles

54%

Of studies cannot be verified

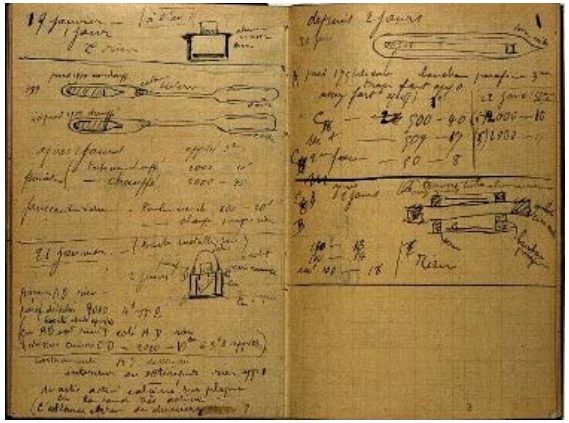


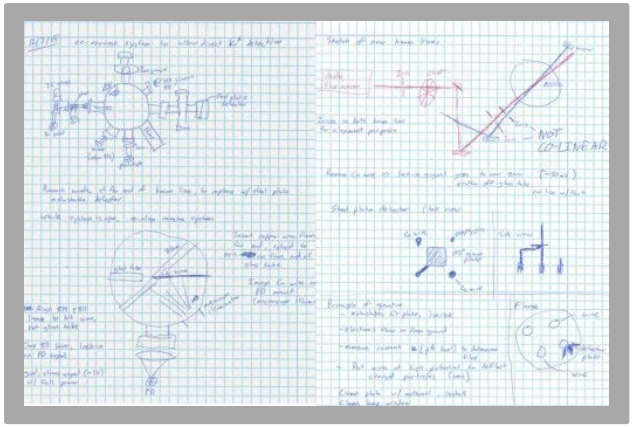
17%

Of scientific data is lost each year

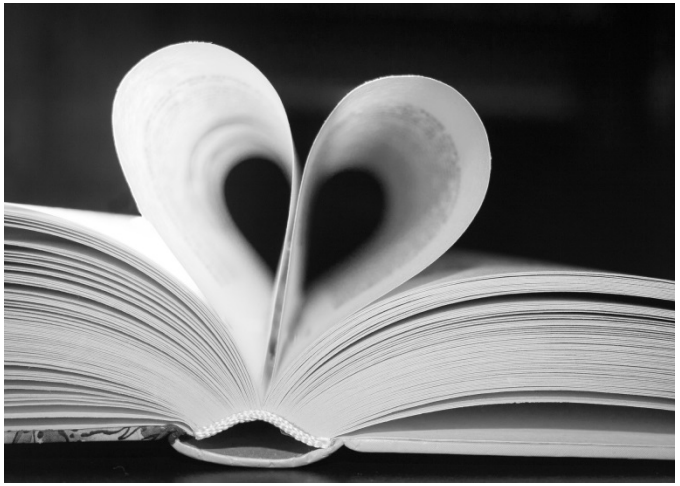
SCINOTE

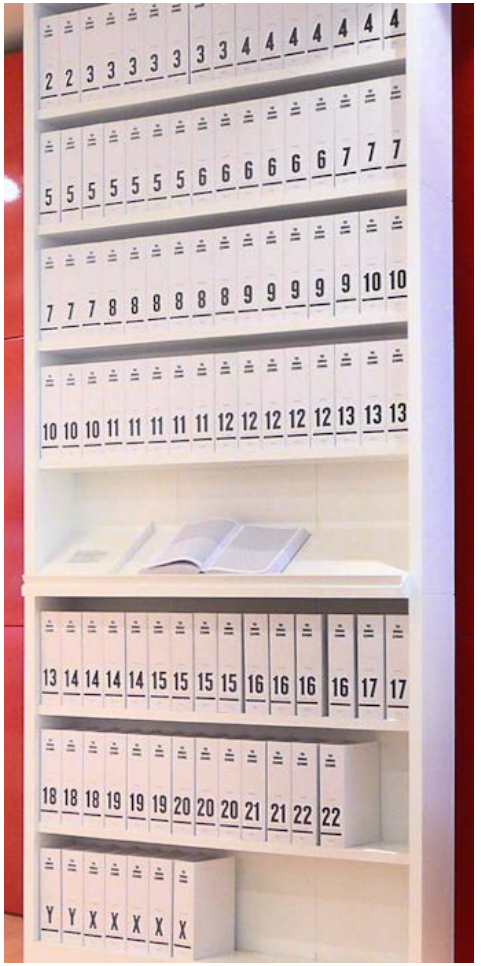
Research: 1900s vs. 2000s





WE ❤ PAPER

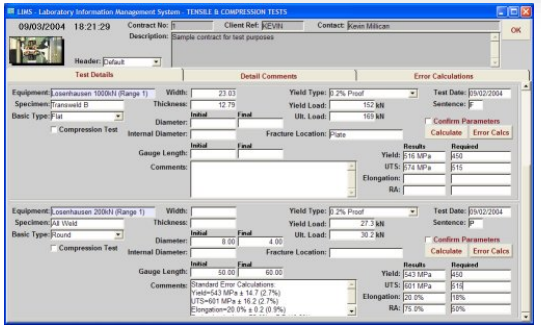




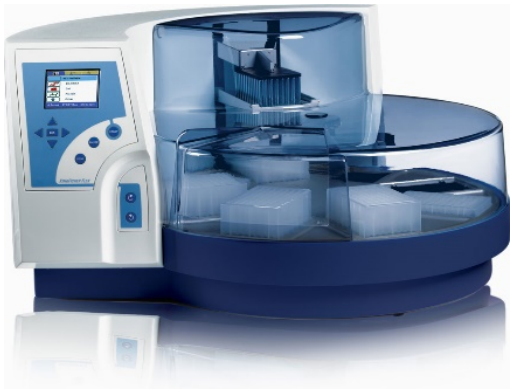
Diagnostic setting – early automation

High throughput → first to automate

Automate with software – LIS, LIMS



Automate with instruments

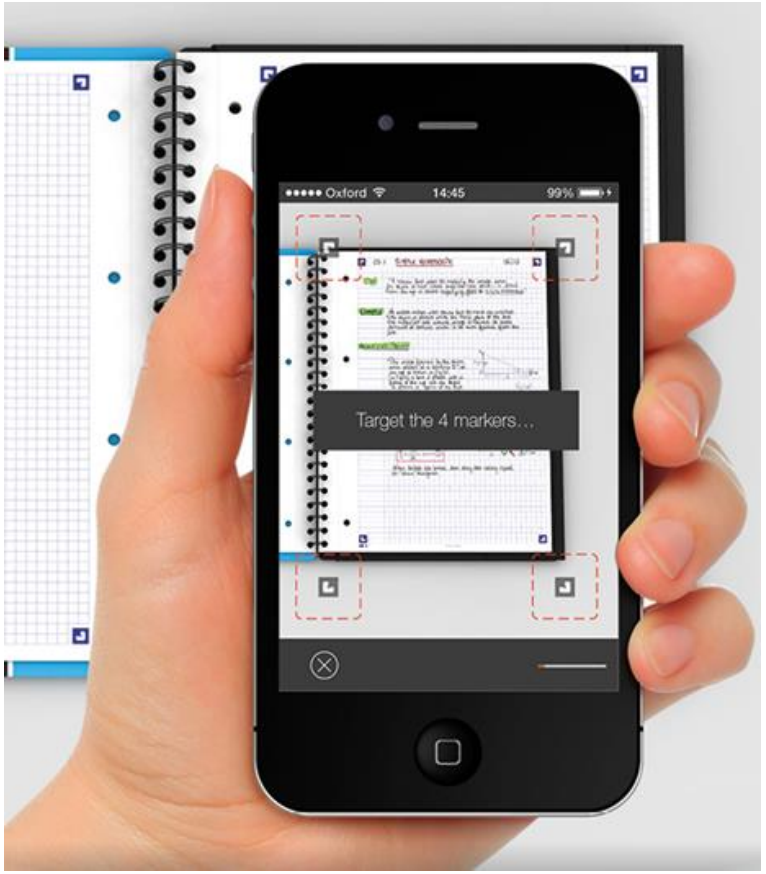


Automation in research?

Generally less throghput → less pressure to automate

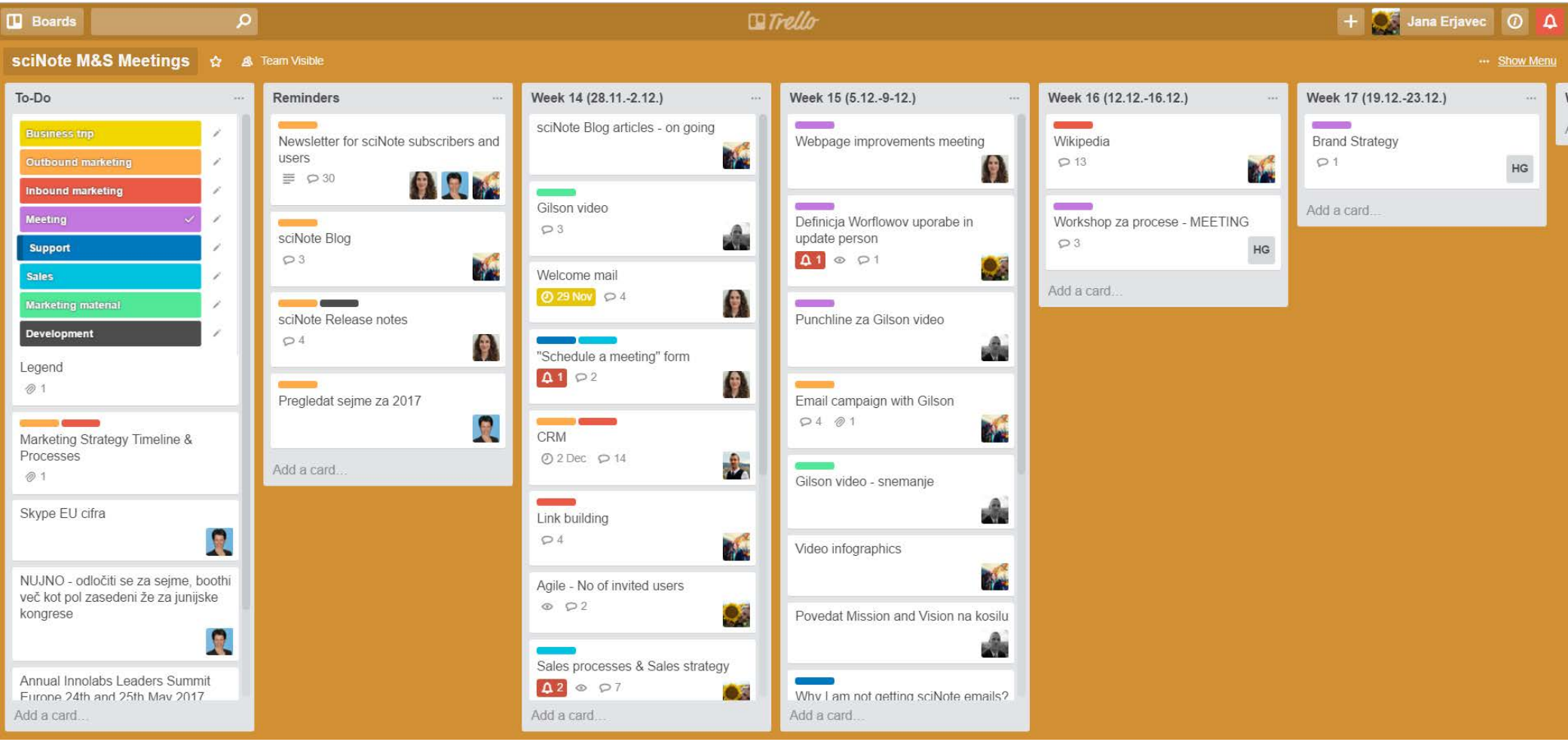
Instruments – same as diagnostics

DIGITISATION

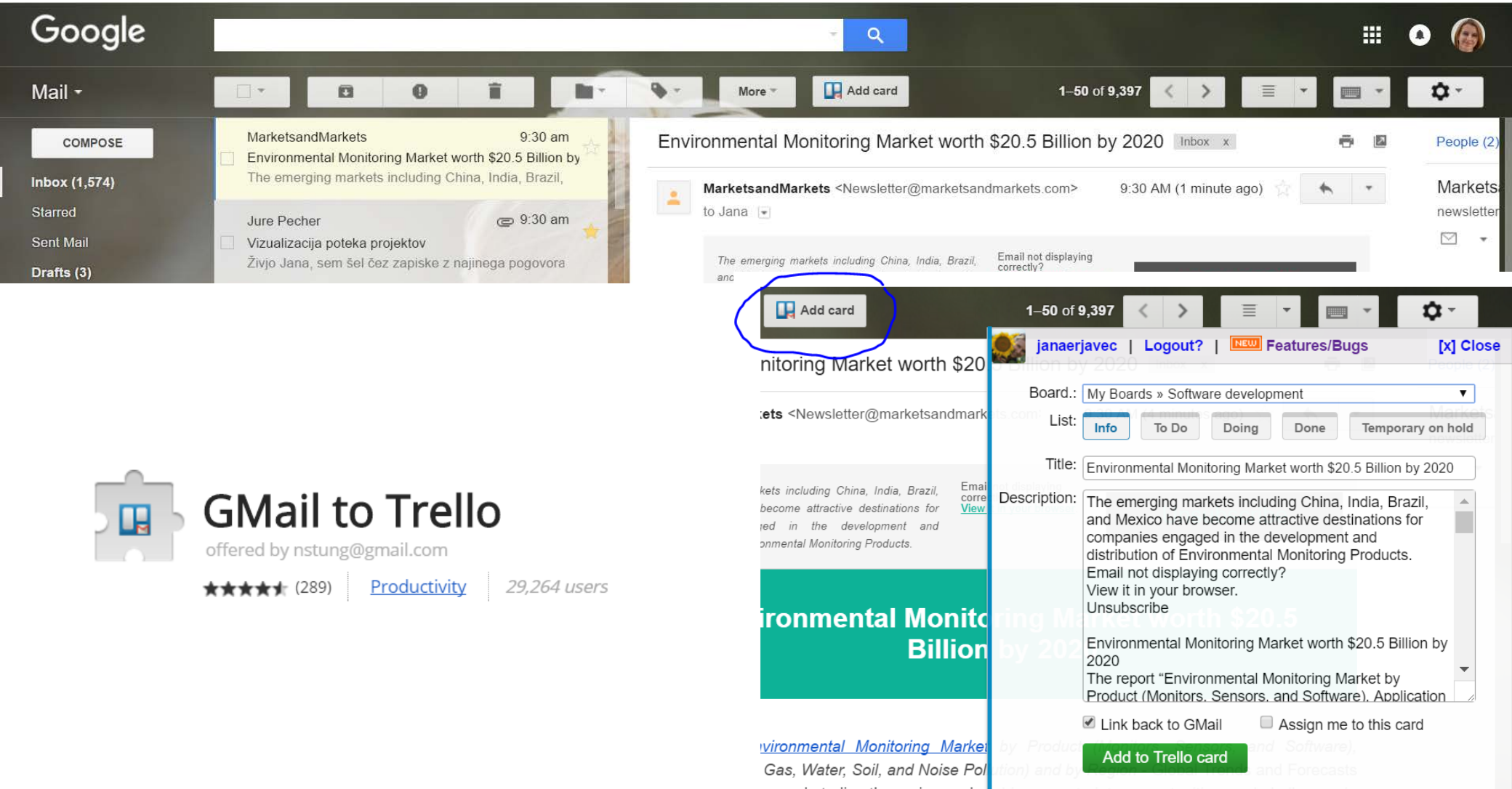


LABORATORY DIGITISATION

LABORATORY DIGITISATION



LABORATORY DIGITISATION



GMail to Trello

offered by nstung@gmail.com

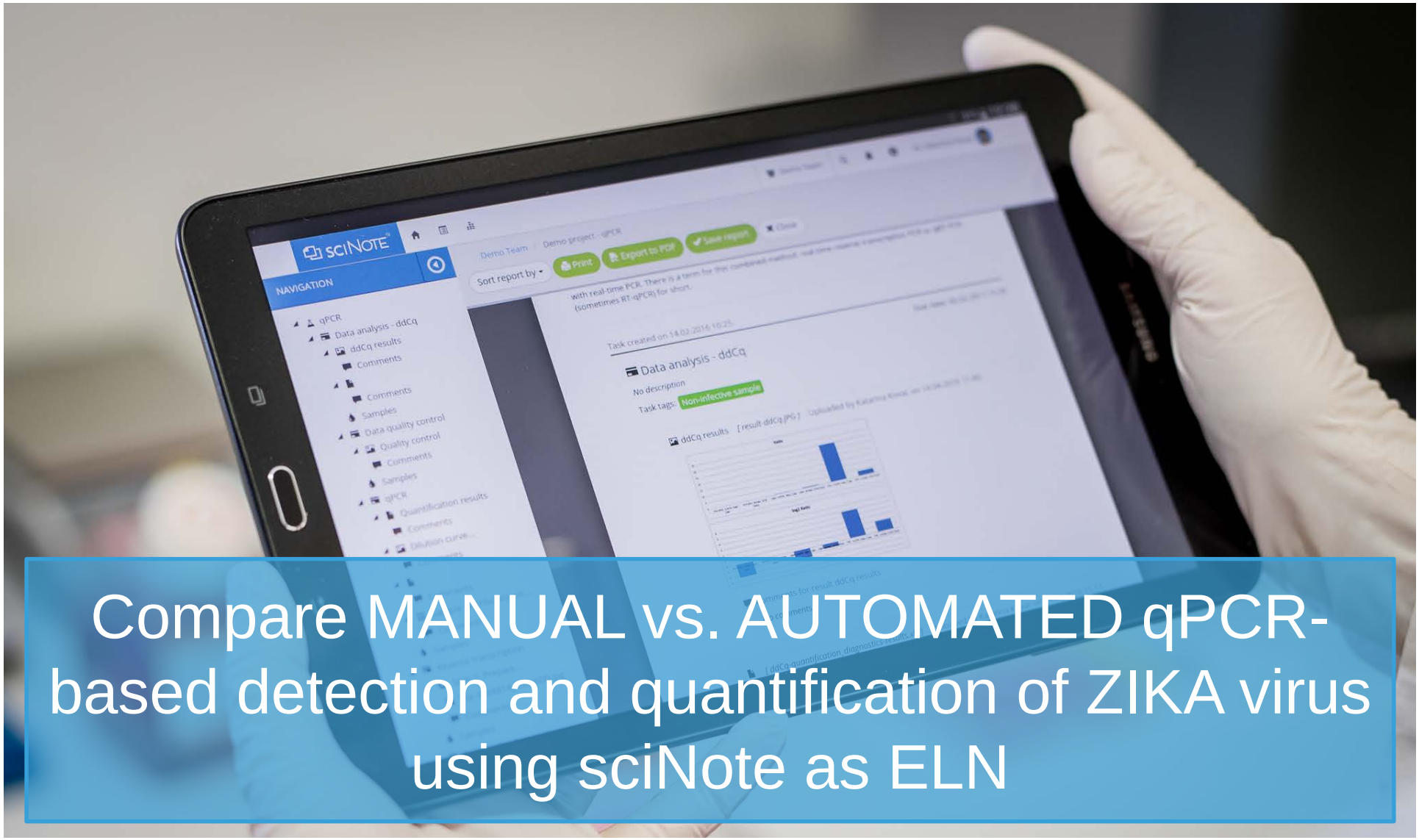
★★★★½ (289) | Productivity | 29,264 users

LABORATORY DIGITISATION

BOOMERANG FOR GMAIL

zapier

Google Sheets
Gmail
Trello
Google Calendar
Slack
MailChimp
Evernote
Twitter
Typeform
Facebook Pages
Google Drive
Asana
Dropbox
PREMIUM
Salesforce
RSS by Zapier
Webhooks by Zapier


Compare MANUAL vs. AUTOMATED qPCR-based detection and quantification of ZIKA virus using sciNote as ELN

MOTIVATION

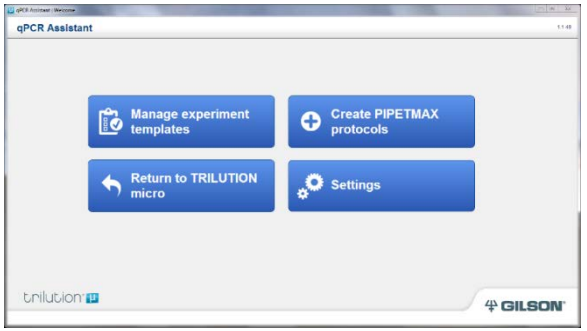
Automate of qPCR-based ZIKV detection and quantification on two levels:

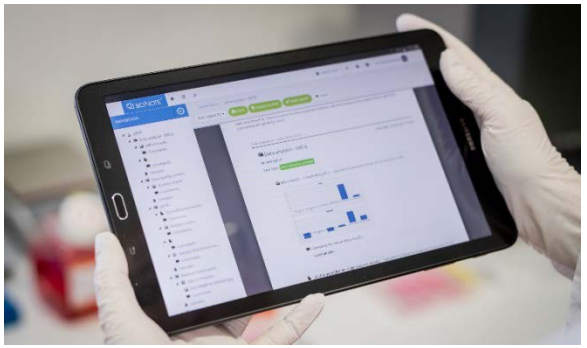
1. Using a robot (PIPETMAX® 268 automated pipetting workstation with qPCR assistant, Gilson Inc)
   & compared to manual pipetting

2. Using sciNote Open Source Electronic Lab Notebook (sciNote LLC) – to increase traceability

Trying to avoid as much paper as possible

Perform experiments in the lab that confirmed the link between ZIKV & Microcephaly





ZIKA VIRUS IS ASSOCIATED with MICROCEPHALY

1947

mosquito-borne flavivirus, isolated from a rhesus monkey in the Zika forest in Uganda

1968-1981

sporadic cases were reported in Southeast Asia and sub-Saharan Africa

2007

outbreak in Yap Island of Micronesia

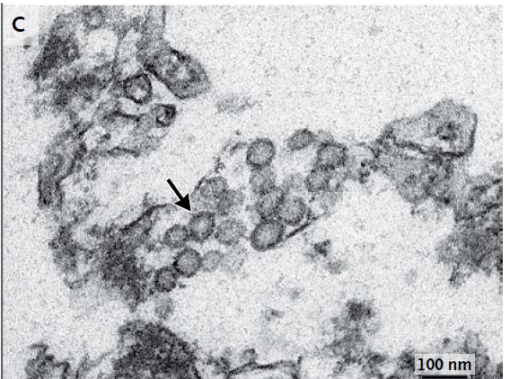
2013/14

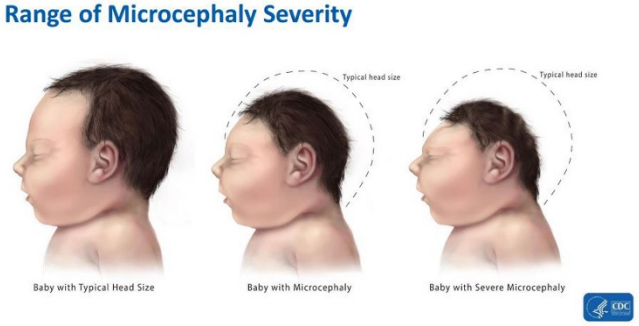
epidemics in French Polynesia, New Caledonia, the Cook Islands, and Easter Island

2016

confirmed association ZIKV & Microcephaly

The NEW ENGLAND JOURNAL of MEDICINE



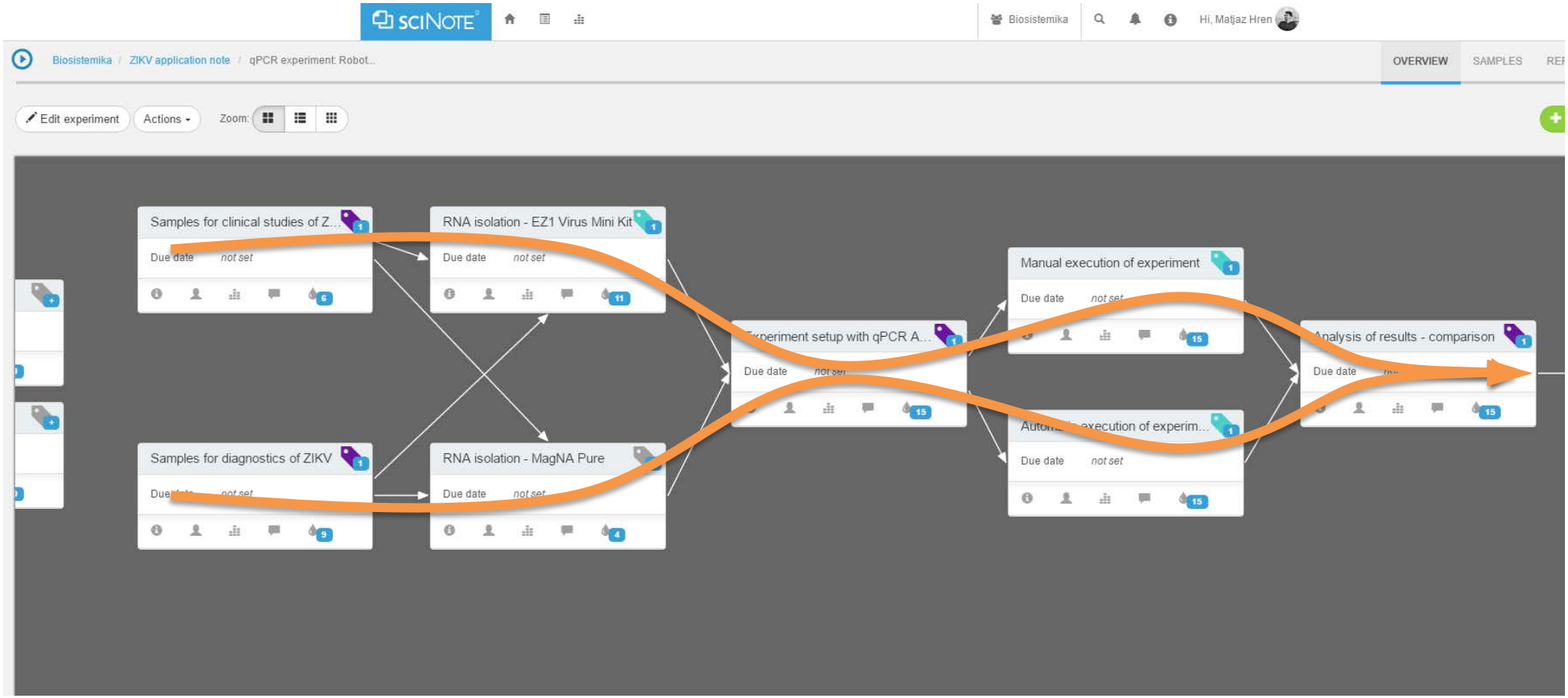


BRIEF REPORT

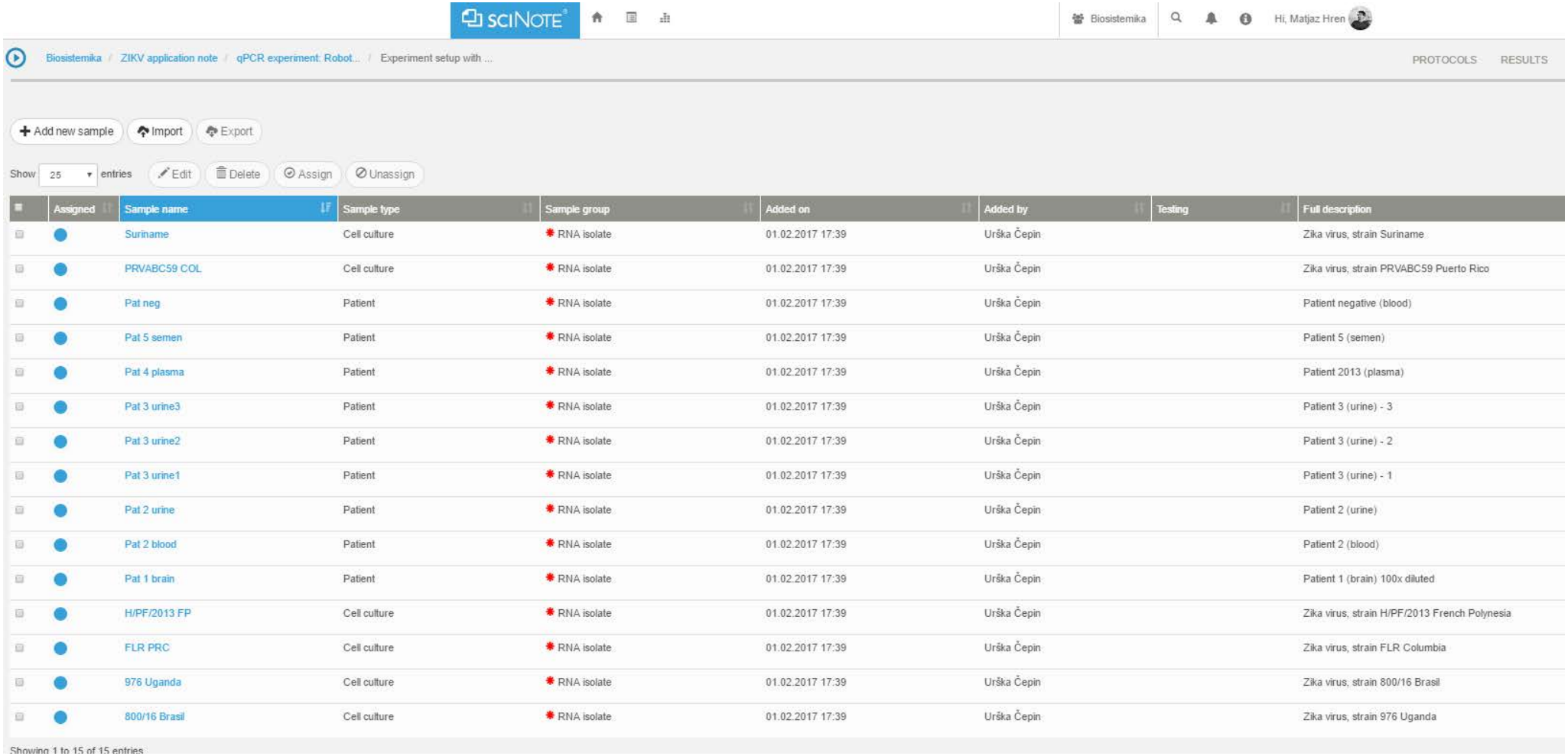
Zika Virus Associated with Microcephaly

Jernej Mlakar, M.D., Misa Korva, Ph.D., Nataša Tul, M.D., Ph.D., Mara Popović, M.D., Ph.D., Mateja Poljšak-Prijatelj, Ph.D., Jerica Mraz, M.Sc., Marko Kolenc, M.Sc., Katarina Resman Rus, M.Sc., Tina Vesnaver Vipotnik, M.D., Vesna Fabjan Vodušek, M.D., Alenka Vizjak, Ph.D., Jože Pižem, M.D., Ph.D., Miroslav Petrovec, M.D., Ph.D., and Tatjana Avšič Županc, Ph.D.

EXPERIMENTAL DESIGN



SAMPLE LIST in sciNOTE



Assigned	Sample name	Sample type	Sample group	Added on	Added by	Testing	Full description
●	Suriname	Cell culture	RNA isolate	01.02.2017 17:39	Urška Čepin		Zika virus, strain Suriname
●	PRVABC59 COL	Cell culture	RNA isolate	01.02.2017 17:39	Urška Čepin		Zika virus, strain PRVABC59 Puerto Rico
●	Pat neg	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient negative (blood)
●	Pat 5 semen	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 5 (semen)
●	Pat 4 plasma	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 2013 (plasma)
●	Pat 3 urine3	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 3 (urine) - 3
●	Pat 3 urine2	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 3 (urine) - 2
●	Pat 3 urine1	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 3 (urine) - 1
●	Pat 2 urine	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 2 (urine)
●	Pat 2 blood	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 2 (blood)
●	Pat 1 brain	Patient	RNA isolate	01.02.2017 17:39	Urška Čepin		Patient 1 (brain) 100x diluted
●	H/PF/2013 FP	Cell culture	RNA isolate	01.02.2017 17:39	Urška Čepin		Zika virus, strain H/PF/2013 French Polynesia
●	FLR PRC	Cell culture	RNA isolate	01.02.2017 17:39	Urška Čepin		Zika virus, strain FLR Columbia
●	976 Uganda	Cell culture	RNA isolate	01.02.2017 17:39	Urška Čepin		Zika virus, strain 800/16 Brasil
●	800/16 Brasil	Cell culture	RNA isolate	01.02.2017 17:39	Urška Čepin		Zika virus, strain 976 Uganda

EXAMPLE OF RNA ISOLATION PROTOCOL in sciNOTE

Biosistemika / ZIKV application note / qPCR experiment: Robot... / RNA isolation - EZ1 Vi...

PROTOCOLS | RESULTS | ACTIVITY | SAMPLES | REPORTS

Start date: **15.02.2017 15:34**

Due date: *not set*

Status: **In progress**

Tags: **Wet lab**

EZ1 Virus Mini Kit v2.0 using EZ1 Advanced XL extraction system (Qiagen) is used for RNA from brain, plasma, blood, semen and cells culture samples.

(unlinked) | Load protocol | Export protocol | Save to protocol repository | Complete task

Protocol steps

Add new | Collapse all | Expand all

3 **Load cartridges, samples and labware into EZ1 instrument and START the instrument** | Published on *30.03.2017 17:06* by *Matjaz Hren*

1. Open the instrument door.
2. Invert reagent cartridges 3 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents to the bottom of their wells.
3. Follow the onscreen instructions for worktable setup, protocol variable selection, and data tracking:
   - **Note**: After sliding a reagent cartridge into the cartridge rack, press down on the cartridge until it clicks into place.
   - **Important**: make sure each elution tube for each sample tube is clearly labelled.
4. Close the instrument door.
5. Press "START" to start the protocol.

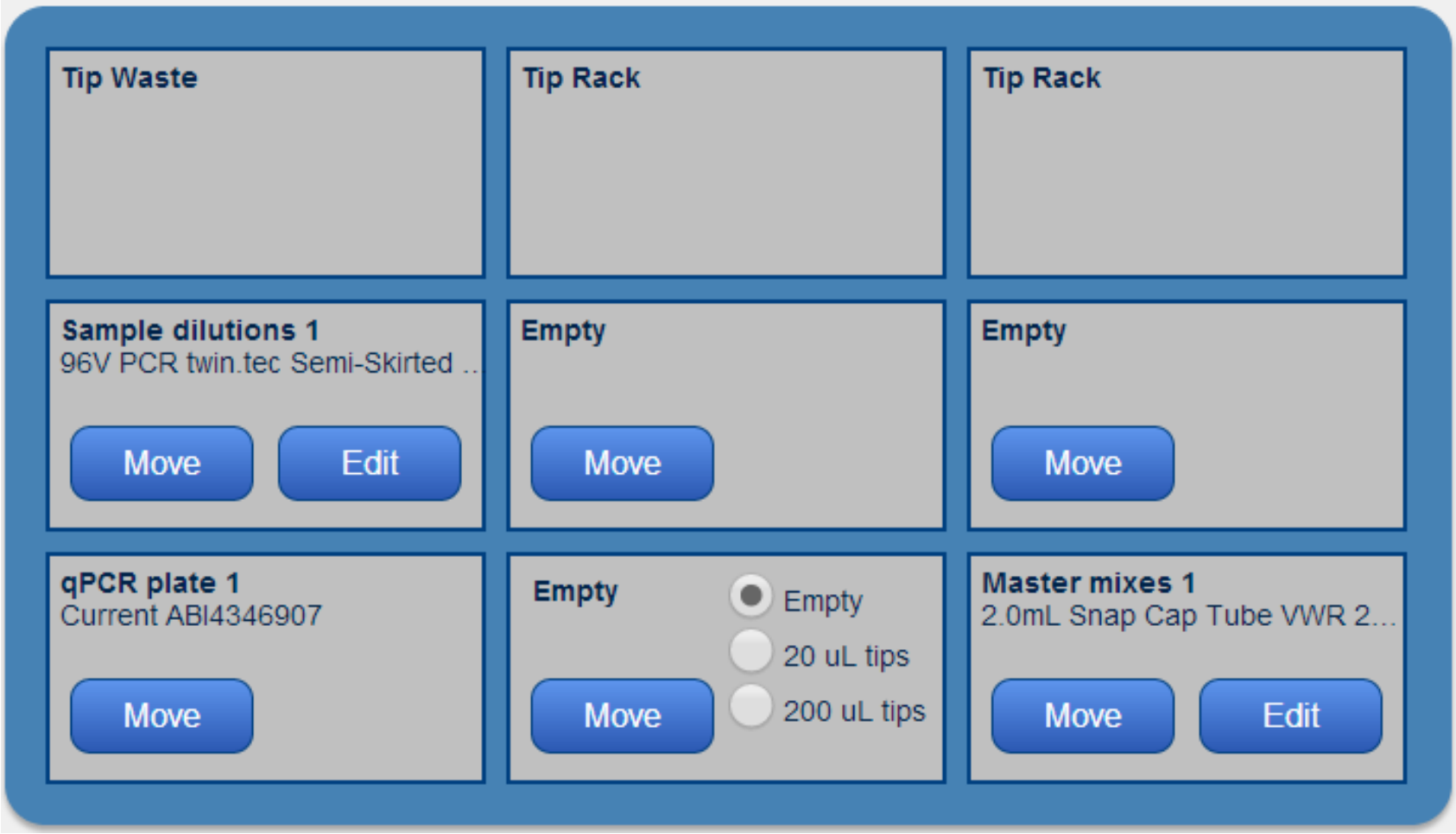
Complete step

Files

qPCR ASSISTANT– AUTO GENERATED qPCR plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	976 Uganda 3p 1.0x	976 Uganda 3p 1.0x	976 Uganda 3p 1.0x	976 Uganda 3p 10.0x	976 Uganda 3p 10.0x	976 Uganda 3p 10.0x	976 Uganda 3p 100.0x	976 Uganda 3p 100.0x	976 Uganda 3p 100.0x	SC1 10^5x	SC1 10^5x	SC1 10^5x
B	800/16 Bra 3p 1.0x	800/16 Bra 3p 1.0x	800/16 Bra 3p 1.0x	800/16 Bra 3p 10.0x	800/16 Bra 3p 10.0x	800/16 Bra 3p 10.0x	800/16 Bra 3p 100.0x	800/16 Bra 3p 100.0x	800/16 Bra 3p 100.0x	SC1 10^6x	SC1 10^6x	SC1 10^6x
C	FLR PRC 1p 1.0x	FLR PRC 1p 1.0x	FLR PRC 1p 1.0x	FLR PRC 1p 10.0x	FLR PRC 1p 10.0x	FLR PRC 1p 10.0x	FLR PRC 1p 100.0x	FLR PRC 1p 100.0x	FLR PRC 1p 100.0x	Pat 2013 plaz 1.0x	Pat 2013 plaz 1.0x	Pat 3 uri3 1.0x
D	H/PF/2013 6p 1.0x	H/PF/2013 6p 1.0x	H/PF/2013 6p 1.0x	H/PF/2013 6p 10.0x	H/PF/2013 6p 10.0x	H/PF/2013 6p 10.0x	H/PF/2013 6p 100.0x	H/PF/2013 6p 100.0x	H/PF/2013 6p 100.0x	Pat 1 brain 1.0x	Pat 1 brain 1.0x	Pat 5 spe 1.0x
E	PRVABC59 COL1p 1.0x	PRVABC59 COL1p 1.0x	PRVABC59 COL1p 1.0x	PRVABC59 COL1p 10.0x	PRVABC59 COL1p 10.0x	PRVABC59 COL1p 10.0x	PRVABC59 COL1p 100.0x	PRVABC59 COL1p 100.0x	PRVABC59 COL1p 100.0x	Pat 2 blood 1.0x	Pat 2 blood 1.0x	Pat neg 1.0x
F	Suriname 5p 1.0x	Suriname 5p 1.0x	Suriname 5p 1.0x	Suriname 5p 10.0x	Suriname 5p 10.0x	Suriname 5p 10.0x	Suriname 5p 100.0x	Suriname 5p 100.0x	Suriname 5p 100.0x	Pat 2 uri 1.0x	Pat 2 uri 1.0x	Pat 3 uri3 1.0x
G	NTC1 1.0x	NTC1 1.0x	NTC1 1.0x	SC1 10.0x	SC1 10.0x	SC1 10.0x	SC1 1000.0x	SC1 1000.0x	SC1 1000.0x	Pat 3 uri1 1.0x	Pat 3 uri1 1.0x	Pat 5 spe 1.0x
H	SC1 1.0x	SC1 1.0x	SC1 1.0x	SC1 100.0x	SC1 100.0x	SC1 100.0x	SC1 10^4x	SC1 10^4x	SC1 10^4x	Pat 3 uri2 1.0x	Pat 3 uri2 1.0x	Pat neg 1.0x

qPCR Assistant – layout of PIPETMAX tray for qPCR plate



qPCR Assistant – sample dilutions

	1	2	3	4	5	6	7	8	9	10	11	12
A	976 Uganda 3p 23,50 µL 1.0x	Pat 3 uri3 17,60 µL 1.0x	976 Uganda 3p 36,00 µL 10.0x	976 Uganda 3p 40,00 µL 100.0x	SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 36,00 µL 10^5x							
B	800/16 Bra 3p 23,50 µL 1.0x	Pat 5 spe 17,60 µL 1.0x	800/16 Bra 3p 36,00 µL 10.0x	800/16 Bra 3p 40,00 µL 100.0x	SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 40,00 µL 10^6x							
C	FLR PRC 1p 23,50 µL 1.0x	Pat neg 17,60 µL 1.0x	FLR PRC 1p 36,00 µL 10.0x	FLR PRC 1p 40,00 µL 100.0x	Pat 2013 plaz 17,60 µL 1.0x							
D	H/PF/2013 6p 23,50 µL 1.0x		H/PF/2013 6p 36,00 µL 10.0x	H/PF/2013 6p 40,00 µL 100.0x	Pat 1 brain 17,60 µL 1.0x							
E	PRVABC59 COL1p 23,50 µL 1.0x		PRVABC59 COL1p 36,00 µL 10.0x	PRVABC59 COL1p 40,00 µL 100.0x	Pat 2 blood 17,60 µL 1.0x							
F	Suriname 5p 23,50 µL 1.0x		Suriname 5p 36,00 µL 10.0x	Suriname 5p 40,00 µL 100.0x	Pat 2 uri 17,60 µL 1.0x							
G	NTC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 23,10 µL 1.0x		SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 36,00 µL 10.0x	SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 36,00 µL 1000.0x	Pat 3 uri1 17,60 µL 1.0x							
H	SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 23,50 µL 1.0x		SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 36,00 µL 100.0x	SC1|ZIKV 1/10/100|ABI 7500 fast 1/10/100 7-log 36,00 µL 10^4x	Pat 3 uri2 17,60 µL 1.0x							

qPCR Assistant – MASTER MIX RECIPES

Master Mix Recipes

Experiment template: 7500ABI-nondiluted_ABI7500Fast_96

Master mix 1: **Assay: ZIKV 1**

Required No. of reactions: **96**

No. of reactions including extra volume: **110.2**

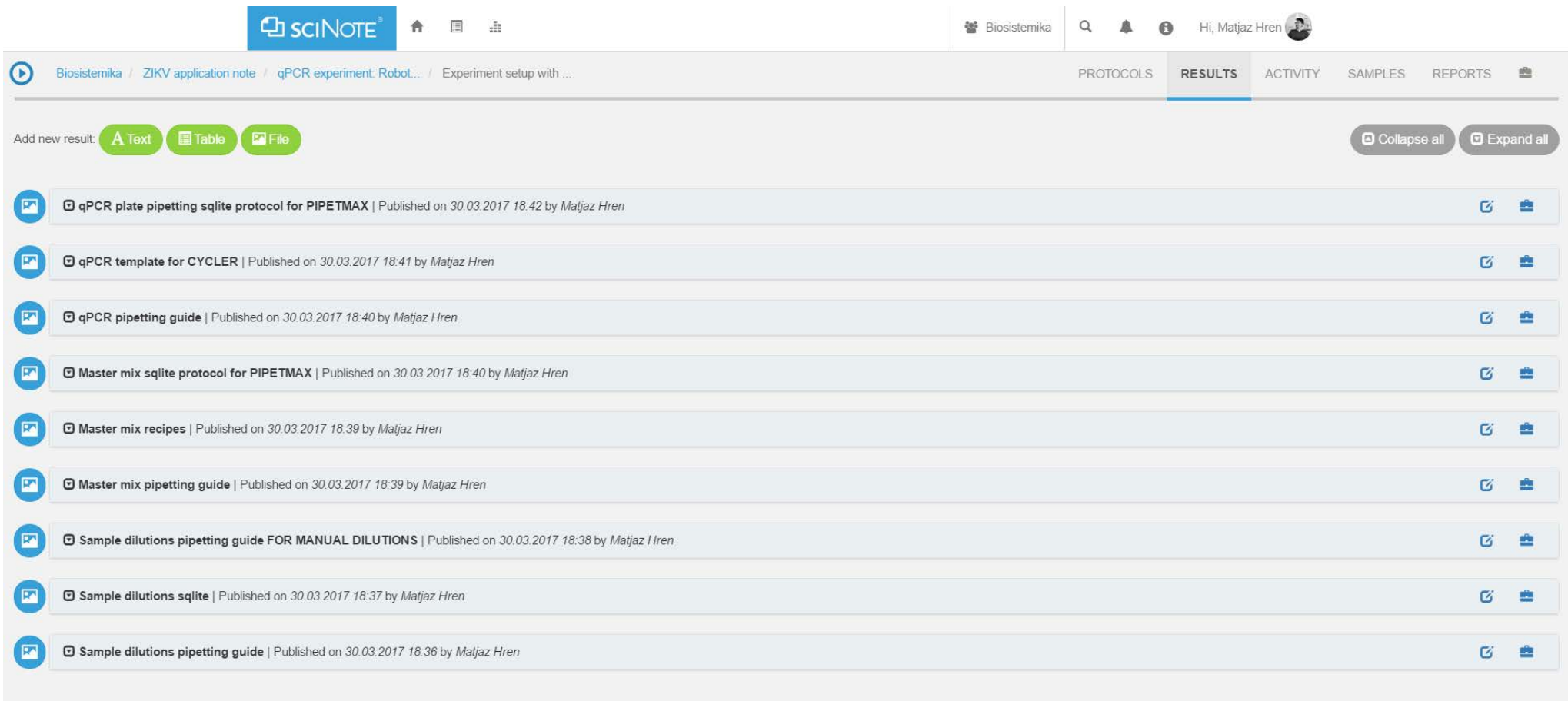
No. of tubes:**1**

Reagent	Initial conc.	Final conc. / reaction	Number of reactions (µL)	
			1	110.2
water	-	-	9.2	**1014.3**
TaqMan Fast Virus 1-Step	4.0 x	1.0 x	5.0	**551.2**
BonnNS1 F	50.0 µM	0.63 µM	0.2	**27.6**
BonnNS1 R	50.0 µM	0.63 µM	0.2	**27.6**
BonnNS1 S	20.0 µM	0.3 µM	0.3	**33.1**
		Total mix volume:	15.0 µL	1653.7 µL
		Sample volume:	5.0 µL	
		Total reaction volume:	20.0 µL	

qPCR Assistant – qPCR TEMPLATE

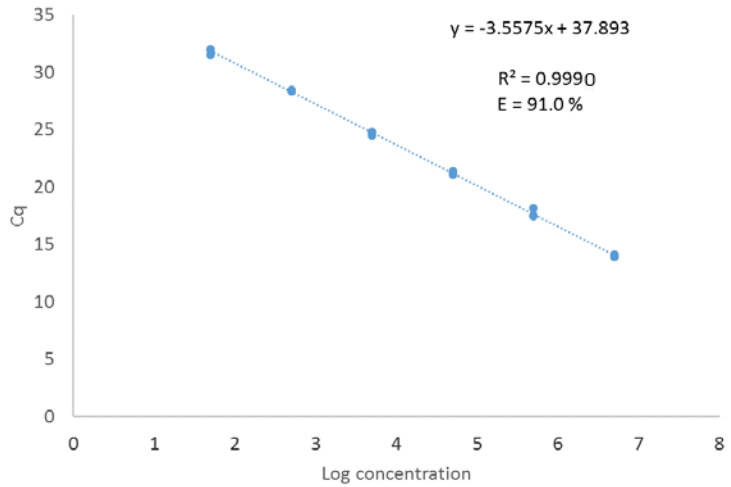
	1	2	3	4	5	6	7	8	9	10	11	12
A	976 Uganda 3p/1.0 U ZIKV 1/10/100	976 Uganda 3p/1.0 U ZIKV 1/10/100	976 Uganda 3p/1.0 U ZIKV 1/10/100	976 Uganda 3p/1... U ZIKV 1/10/100	976 Uganda 3p/1... U ZIKV 1/10/100	976 Uganda 3p/1... U ZIKV 1/10/100	976 Uganda 3p/1... U ZIKV 1/10/100	976 Uganda 3p/1... U ZIKV 1/10/100	976 Uganda 3p/1... U ZIKV 1/10/100	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0
B	800/16 Bra 3p/1.0 U ZIKV 1/10/100	800/16 Bra 3p/1.0 U ZIKV 1/10/100	800/16 Bra 3p/1.0 U ZIKV 1/10/100	800/16 Bra 3p/10.0 U ZIKV 1/10/100	800/16 Bra 3p/10.0 U ZIKV 1/10/100	800/16 Bra 3p/10.0 U ZIKV 1/10/100	800/16 Bra 3p/10... U ZIKV 1/10/100	800/16 Bra 3p/10... U ZIKV 1/10/100	800/16 Bra 3p/10... U ZIKV 1/10/100	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0
C	FLR PRC 1p/1.0 U ZIKV 1/10/100	FLR PRC 1p/1.0 U ZIKV 1/10/100	FLR PRC 1p/1.0 U ZIKV 1/10/100	FLR PRC 1p/10.0 U ZIKV 1/10/100	FLR PRC 1p/10.0 U ZIKV 1/10/100	FLR PRC 1p/10.0 U ZIKV 1/10/100	FLR PRC 1p/100.0 U ZIKV 1/10/100	FLR PRC 1p/100.0 U ZIKV 1/10/100	FLR PRC 1p/100.0 U ZIKV 1/10/100	Pat 2013 plaz/1.0 U ZIKV 1	Pat 2013 plaz/1.0 U ZIKV 1	Pat 3 uri3/1.0 U ZIKV 1
D	H/PF/2013 6p/1.0 U ZIKV 1/10/100	H/PF/2013 6p/1.0 U ZIKV 1/10/100	H/PF/2013 6p/1.0 U ZIKV 1/10/100	H/PF/2013 6p/10.0 U ZIKV 1/10/100	H/PF/2013 6p/10.0 U ZIKV 1/10/100	H/PF/2013 6p/10.0 U ZIKV 1/10/100	H/PF/2013 6p/10... U ZIKV 1/10/100	H/PF/2013 6p/10... U ZIKV 1/10/100	H/PF/2013 6p/10... U ZIKV 1/10/100	Pat 1 brain/1.0 U ZIKV 1	Pat 1 brain/1.0 U ZIKV 1	Pat 5 spe/1.0 U ZIKV 1
E	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	PRVABC59 COL1... U ZIKV 1/10/100	Pat 2 blood/1.0 U ZIKV 1	Pat 2 blood/1.0 U ZIKV 1	Pat neg/1.0 U ZIKV 1
F	Suriname 5p/1.0 U ZIKV 1/10/100	Suriname 5p/1.0 U ZIKV 1/10/100	Suriname 5p/1.0 U ZIKV 1/10/100	Suriname 5p/10.0 U ZIKV 1/10/100	Suriname 5p/10.0 U ZIKV 1/10/100	Suriname 5p/10.0 U ZIKV 1/10/100	Suriname 5p/100.0 U ZIKV 1/10/100	Suriname 5p/100.0 U ZIKV 1/10/100	Suriname 5p/100.0 U ZIKV 1/10/100	Pat 2 uri/1.0 U ZIKV 1	Pat 2 uri/1.0 U ZIKV 1	Pat 3 uri3/1.0 U ZIKV 1
G	NTC1|ZIKV 1/10/... N ZIKV 1/10/100	NTC1|ZIKV 1/10/... N ZIKV 1/10/100	NTC1|ZIKV 1/10/... N ZIKV 1/10/100	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0.1	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0.1	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0.1	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	Pat 3 uri1/1.0 U ZIKV 1	Pat 3 uri1/1.0 U ZIKV 1	Pat 5 spe/1.0 U ZIKV 1
H	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 1	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 1	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 1	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0.01	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0.01	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0.01	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	SC1|ZIKV 1/10/10... S ZIKV 1/10/100 0	Pat 3 uri2/1.0 U ZIKV 1	Pat 3 uri2/1.0 U ZIKV 1	Pat neg/1.0 U ZIKV 1

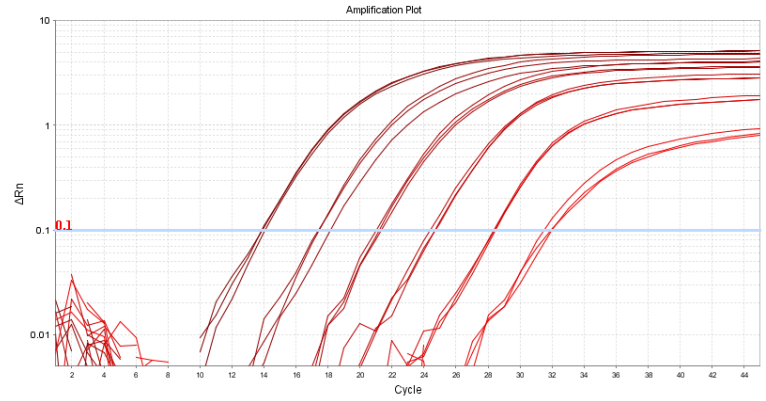
qPCR Assistant FILES UPLOADED TO sciNote



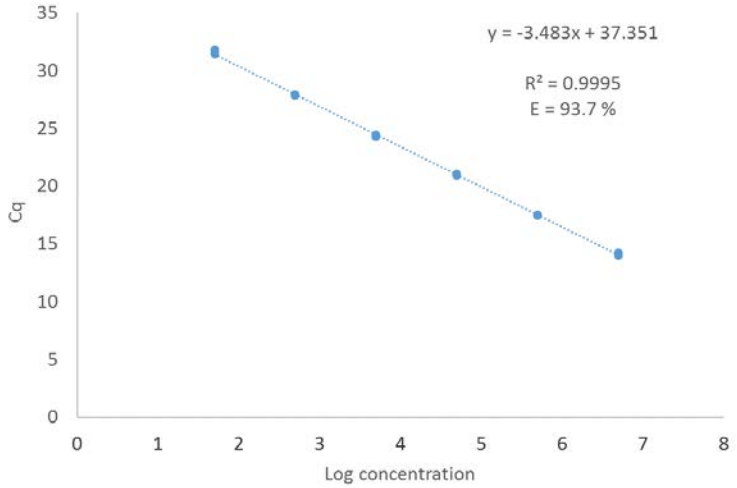
RESULTS – MANUAL vs. ROBOT STANDARD CURVE

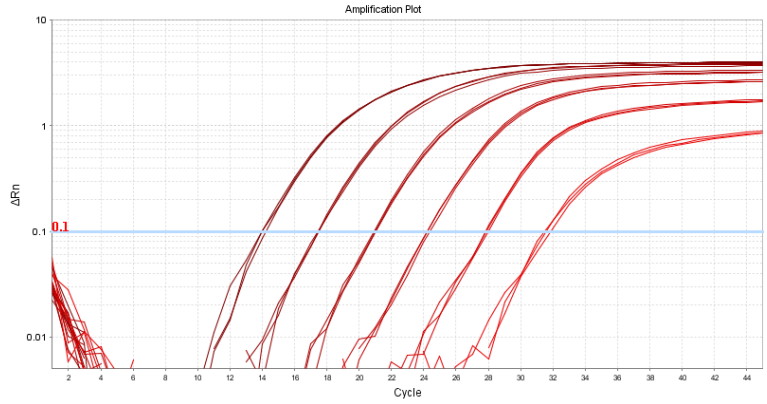
MANUAL



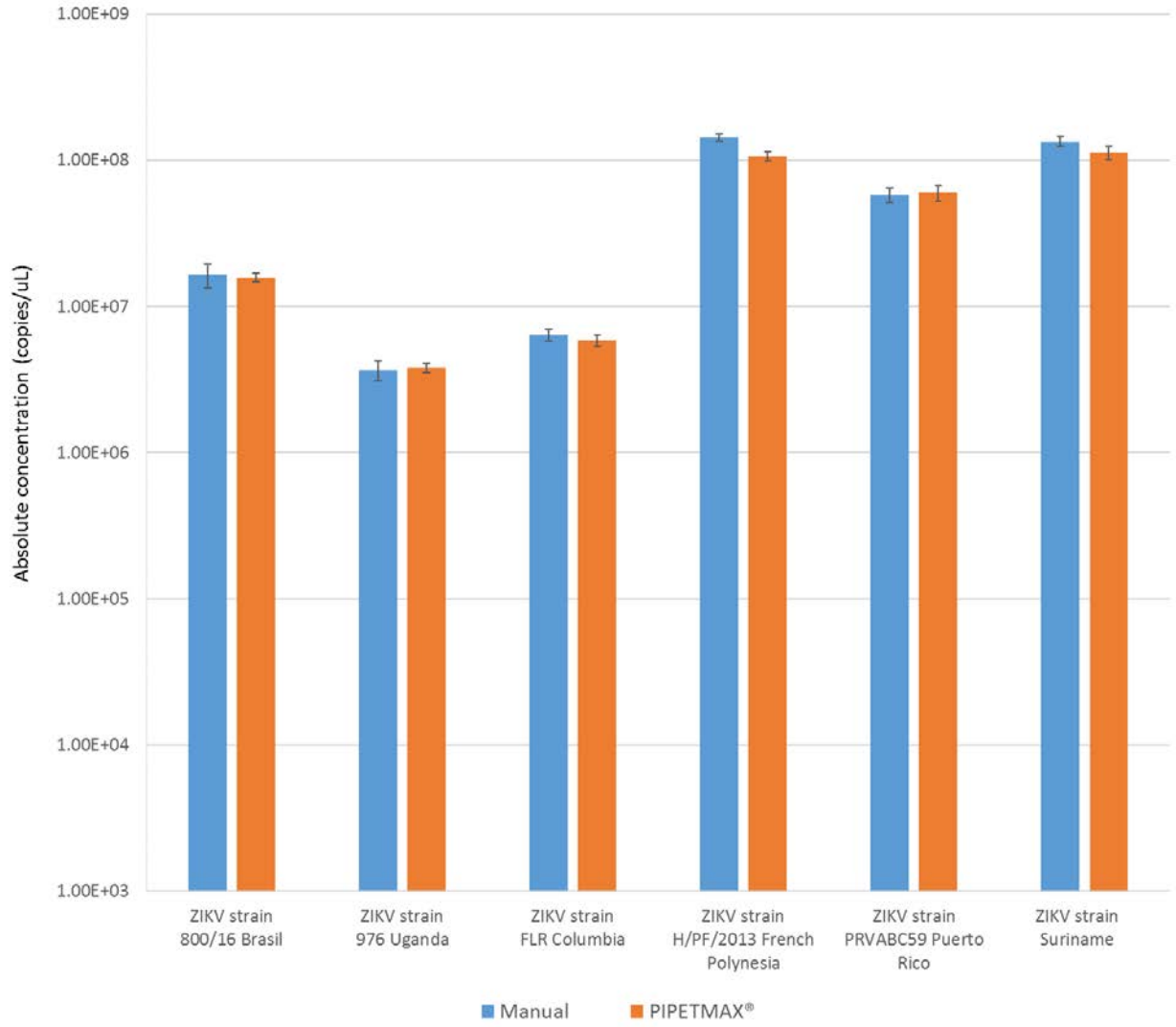


ROBOT





RESULTS – MANUAL vs. ROBOT ZIKV QUANTIFICATION

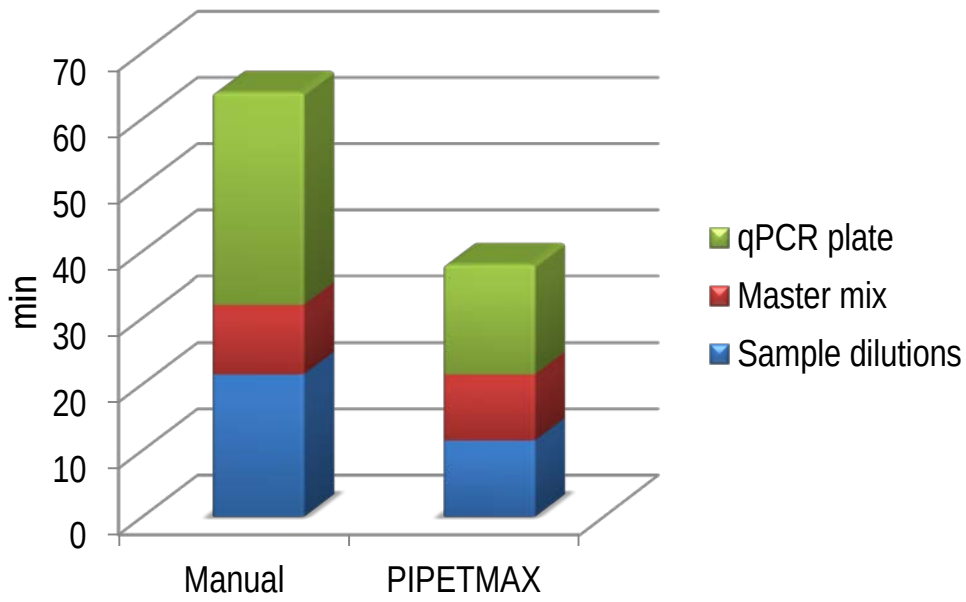


RESULTS – MANUAL vs. ROBOT ZIKV DETECTION

	Manual	PIPETMAX	Manual	PIPETMAX	Delta
Sample Name	Cq	Cq	AVG(Cq)	AVG(Cq)	Delta
Pat 1 brain/100.0	24.96	25.17	25.00	25.02	0.03
Pat 1 brain/100.0	25.03	24.88			
Pat 2 blood/1.0	32.62	34.45	32.78	34.00	1.22
Pat 2 blood/1.0	32.94	33.55			
Pat 2 urine/1.0	33.88	35.21	34.27	35.26	0.99
Pat 2 urine/1.0	34.66	35.31			
Pat 2013 plaz/1.0	33.55	34.58	33.55	36.15	2.60
Pat 2013 plaz/1.0	33.55	37.71			
Pat 3 urine1/1.0	34.06	36.12	34.98	36.94	1.97
Pat 3 urine1/1.0	35.90	37.77			
Pat 3 urine2/1.0	Na	Na		44.14	
Pat 3 urine2/1.0	Na	44.14			
Pat 3 urine3/1.0	Na	Na			
Pat 3 urine3/1.0	Na	Na			
Pat 5 semen/1.0	30.44	30.91	30.44	30.77	0.33
Pat 5 semen/1.0	30.44	30.63			
Pat negative/1.0	Na	Na			
Pat negative/1.0	Na	Na			

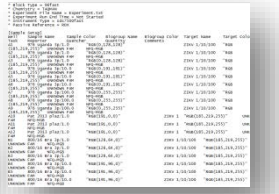
RESULTS – MANUAL vs. ROBOT SPEED

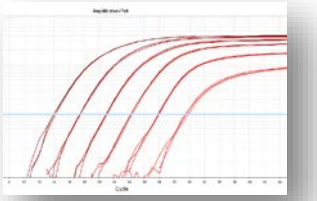
	Manual (min:s)	PIPETMAX (min:s)
Sample dilutions	21:30	11:30
Master mix	10:30	10:00
qPCR plate	32:00	16:30
SUM	**64:00**	**38:00**



SUMMARY

PIPETMAX qPCR Assistant

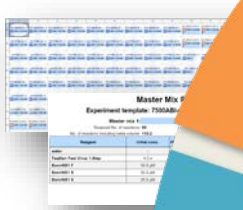


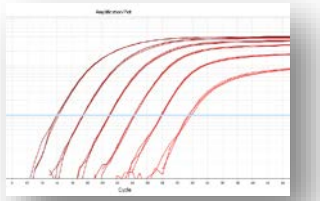


Patient samples	Recipes and pipetting guide	qPCR reaction plate setup	Import run file into thermal cycler	Real-time qPCR	Data analysis
• Sample lists • Patient data and sample tracking	• Auto-calculated volumes •TRILUTION micro qPCR Assistant	• Automated with PIPETMAX® qPCR Assistant v1.3 • vs. manual liquid handling	• Run file generated by qPCR Assistant • Reduces error vs. manual data entry	• Applied Biosystems 7500 • Amplify with TaqMan® Gene Expression Assays	• Quality control checks • Patient diagnosis

SciNote Open source electronic lab notebook

SUMMARY





Patient samples

- Sample lists
- Patient data and sample tracking

- Auto-c
volume
- TRILUTIC
qPCR Ass

00

ne
Assays

Data analysis

- Quality control checks
- Patient diagnosis

PIPETMAX

Closing the gap in traceability

SciNote

SciNote Open source electronic lab notebook

CONCLUSIONS

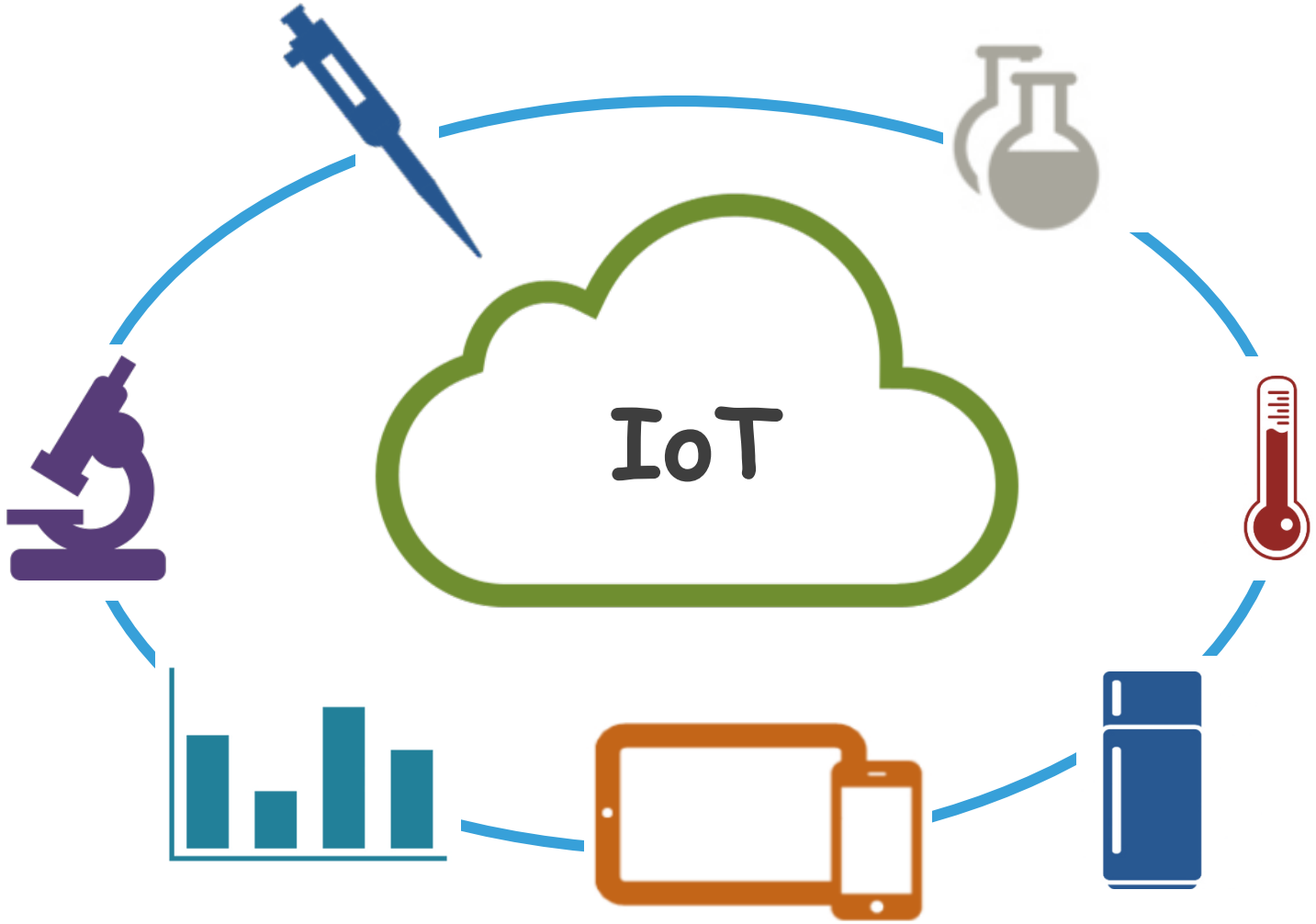
using automation platform

- accurate standard dilution
- assay setup with significant increase in throughput

integration of data management software

- enables full traceability of samples and results → critical for accuracy in human diagnostics:
  - all in one place
  - all actions related to sample are recorded
  - backtrack any deviations/errors
- easier transfer of knowledge
- easier collaboration
- offers basic project management in science
- reduce possibility to lose data

OUTLOOK: BEYOND ROBOTS, BEYOND ELN



ACKNOWLEDGMENTS

Laboratory Automation and Data Management in Diagnostics – qPCR ZIKV Detection and Quantification

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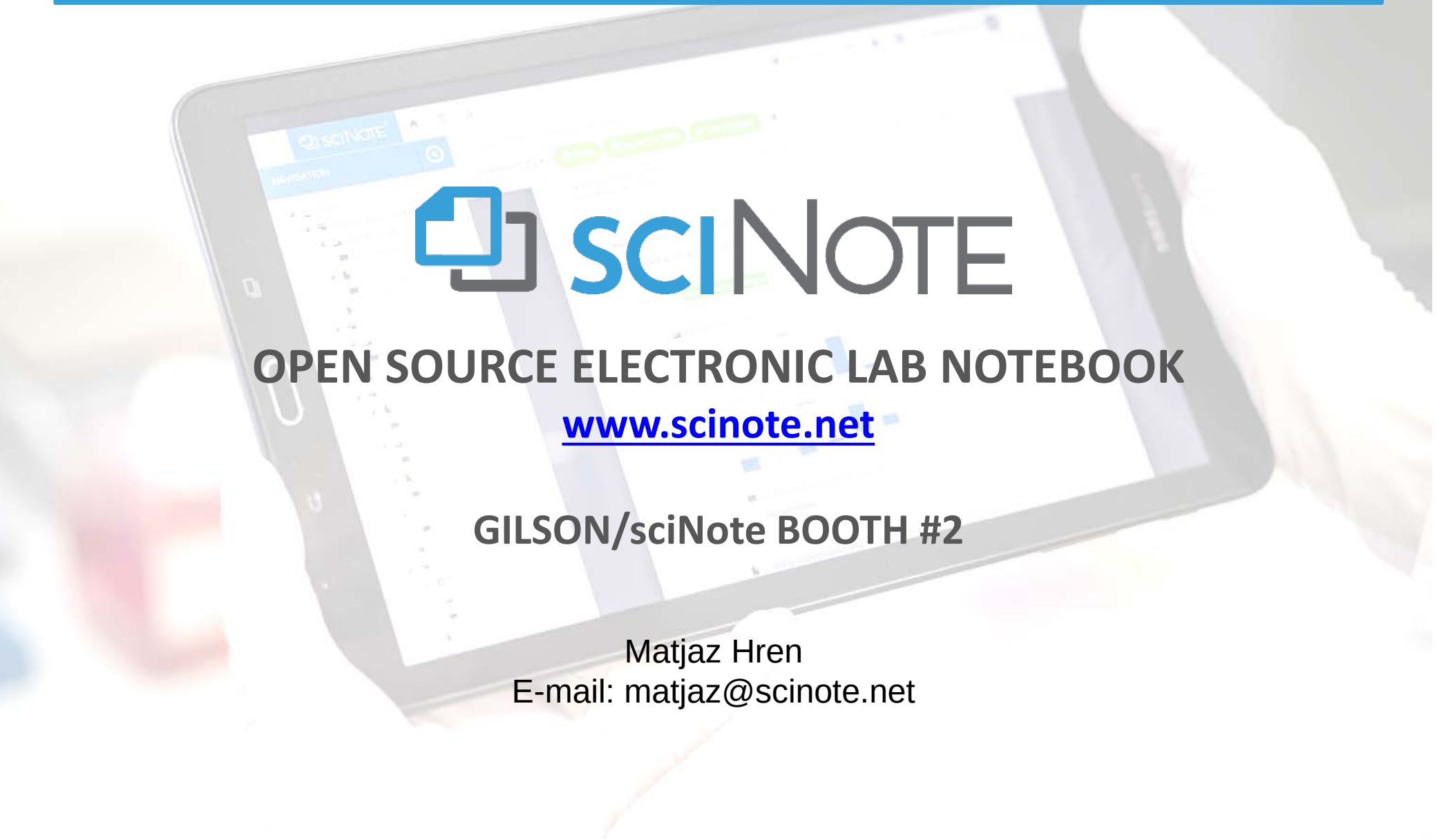
[3]Gilson Inc.

[4]SciNote LLC

THANK YOU!



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