



PROTAK SCIENTIFIC

Enzyme Indicators

Paul Liu PhD

Softbio Health Inc.

晁丞健康股份有限公司

FASTER

SMARTER

SAFER

Agenda

- Company Overview
- Enzyme Indicators
- Current Methodology
- Advantages of EI's
- Application
- Questions



Public Health
England



Global Enzyme Indicator Manufacturer & Supplier
Headquarters in Surrey, UK
Operations & Manufacturing



4 Global Offices
UK, Italy, USA, China



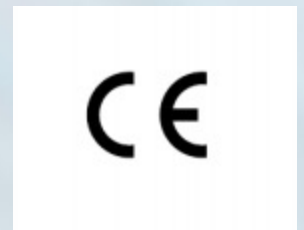
Extensive Distributor Network
Europe, Asia, China and growing



Developed in partnership with PHE
75+ Global Patents, CE & 21 CFR Part 11 Compliant



Rapid Growth & Adoption





Over 50% of top 20 Pharma Companies currently investigating EI use



- We are currently in Talks and active trials with 10 of the Worlds top 20 Pharmaceutical companies.
- Many of the top pharmaceutical leaders are already using eBIs to benefit their production.



*The above companies are for illustration purposes only.

Current Technology & Challenges

Conventional Biological Indicators



Extended Wait

3 DAYS, 5 DAYS, 7 DAYS?

Incubation for an extended timeframe.

Hampers delivery of new equipment, revalidation and product release



Poor Data

BINARY RESULTS

No linear, numerical values of performance which can generate long term statistical data



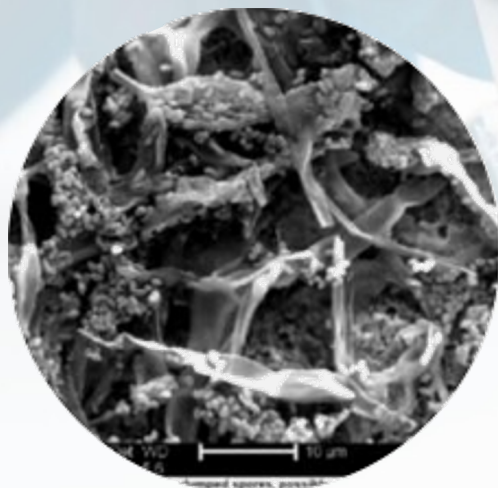
High Process Costs

RUN TO FAIL

Many aspects of Biological Indicator validation place unnecessary costs into the manufacturing process

The challenge today –

When is a Bacillus 10^6 BI *really* a 10^6 BI?



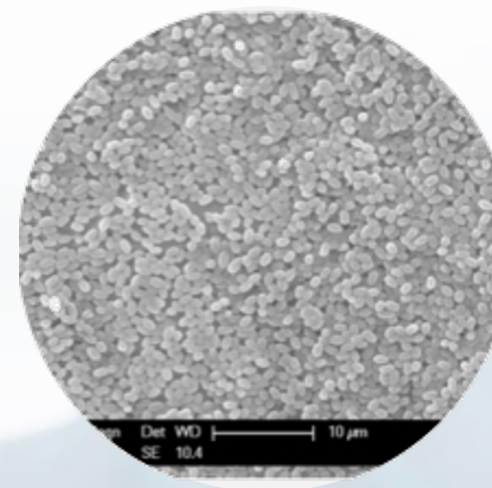
**Contaminated
Carrier**

Physical contamination of
carrier and inoculation



Mass Clumping

Impossible to inoculate,
nucleus surrounded with
shield

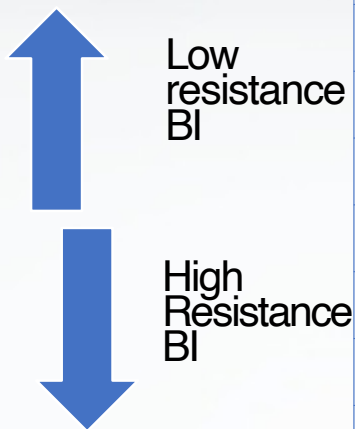


**Bacillus
Composition**

The perfect composition
Impossible to create
consistent challenge.

When is a Bacillus 10^6 BI *really* a 10^6 BI?

D Value	Log 6	Log 5	Log 4	Log 3	Log 2	Log 1		
	1,000,000	100,000	10,000	1,000	100	10	1	
(Time to reduce 1 LOG in minutes)								
Low resistance BI	0.8	0.8	1.6	2.4	3.2	4	4.8	5.6
	1	1	2	3	4	5	6	7
	1.5	1.5	3	4.5	6	7.5	9	10.5
High Resistance BI	2	2	4	6	8	10	12	14
	2.5	2.5	5	7.5	10	12.5	15	17.5
	2.8	2.8	5.6	8.4	11.2	14	16.8	19.6
	3	3	6	9	12	15	18	21



**Deactivation
time in minutes**

**The challenge by nature is variable. Design of Challenge Parameters is Key to Cycle Development.
Variance of approx. 300%**

* Low resistance BI vs High Resistance BI (inactivation time in a BI evaluation using a BIER vessel)

Chemical Indicators



- Indicate vH_2O_2 Presence
- Variable colour change to different vH_2O_2 processes
- Cannot be used as proof of log reduction

The Enzymatic Indicator - The EI

Faster, Smarter & Safer Alternative to BI's



Instant

Taking just 60 seconds

NO incubation.

No delay.

Immediate Results



Real Numbers

Quantifiable Data

Quantitative
Numerical Scale
Correlated to BI's



Digital Data

No manual interpretation

Greatly reduced
manual recording
Simple to use



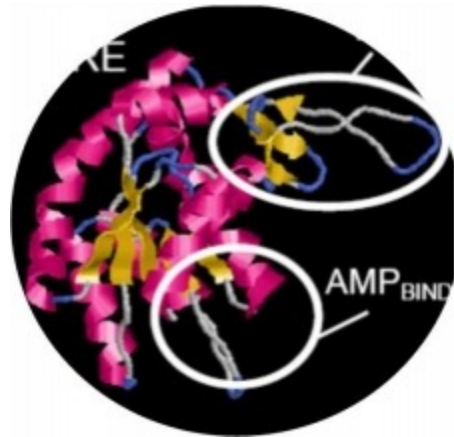
Quality

Built in safeguards

Continuous checking
and validation of EI's
Positive and
Negative Controls



thermostable Adenylate Kinase – The Catalyst



tAK

The Enzyme

Thermally stable and denatures in a remarkably predictable way. Time and dose responsive to oxidization processes.

Highly Compressed Ribbon. Hard to quickly inactive

Resistance to temperature

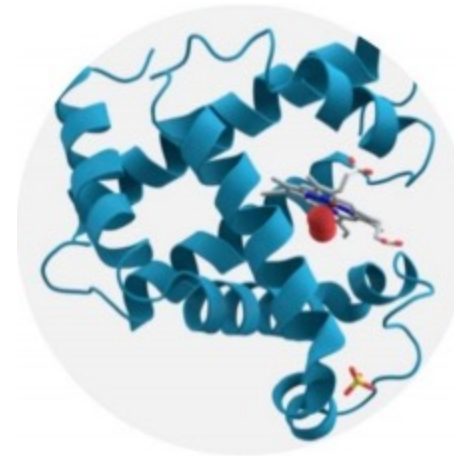
Excellent

Oxidization Processes

Excellent

Stability at 4°C

Excellent



AK

Open ribbon, almost immediately inactivates with low doses of any oxidization processes.

Resistance to temperature

Poor

Oxidization Processes

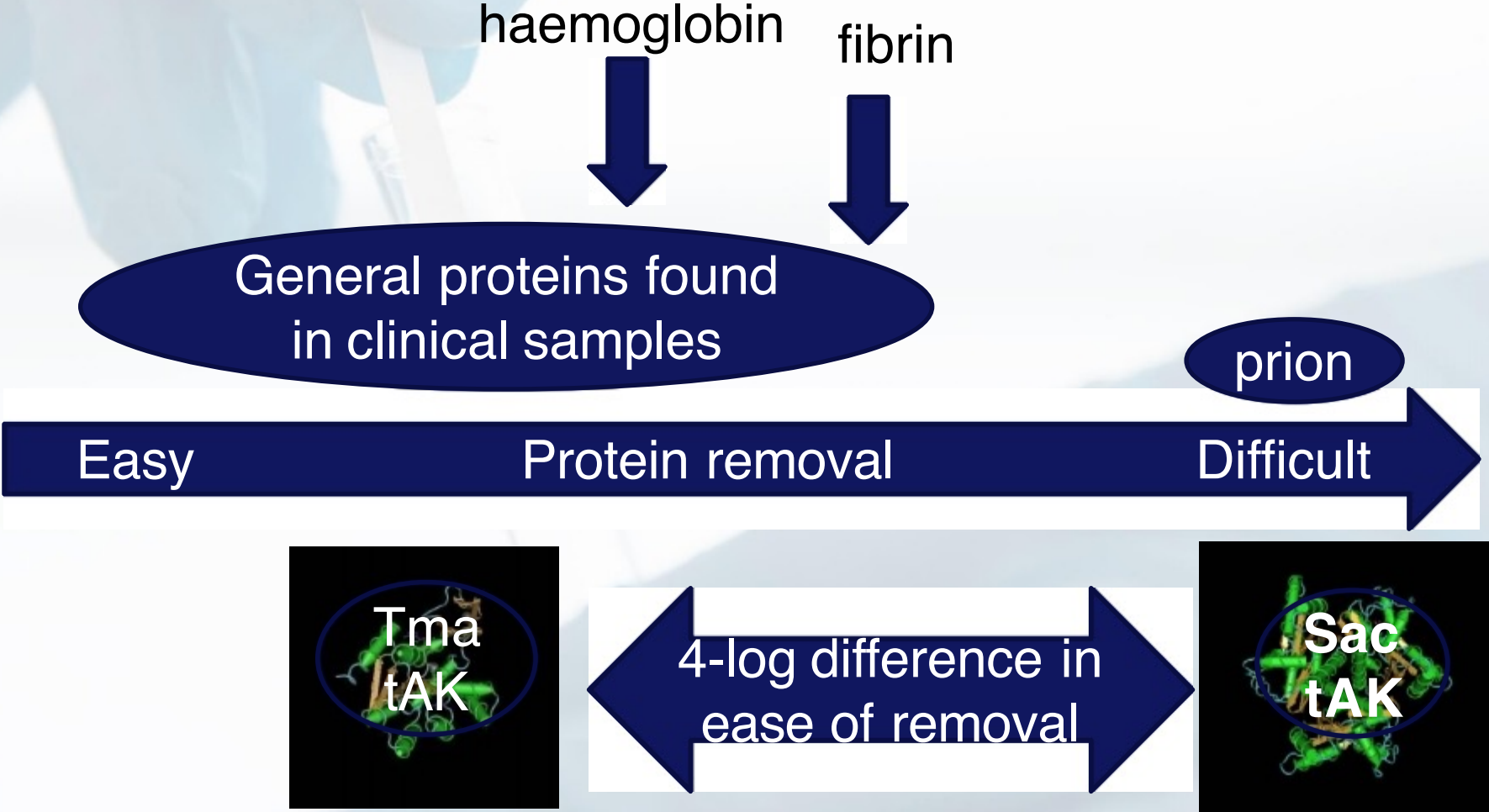
Poor

Stability at 4°C

Okay

tAK is a Biological NON viable so can be used in process

tAK chosen as a protein that adheres tightly to surfaces and represents a worst-case



Enzymatic Technology

1



Enzyme Indicator

tAK Catalyst

Engineered quantity of Enzyme deposited to carrier. Viability reduced to exposure to oxidization processes

2



The Reaction

Bioluminescent Assay

Assay function dependent upon viable enzyme introduced from EI test strip

3



The Reader

Luminometer PR2A

Accurately and Repeatedly measures light in RLU from Enzyme driven luminescent reaction. With inbuilt process qualification

4



The Result

Digital Delivery

Quantitatively, Immediate and Validated cycle efficacy reporting delivered via developed software.

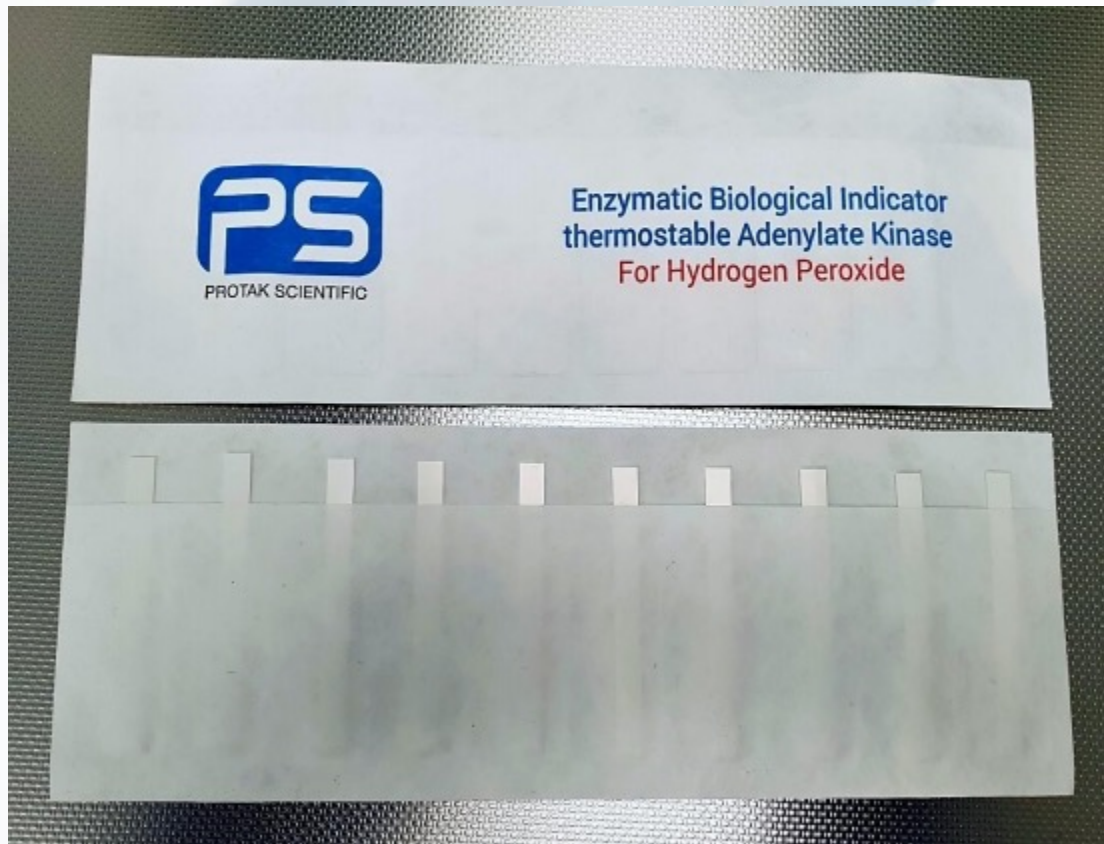
60 seconds

Enzymatic Indicator Strips

- High degree of manufacturing accuracy
- Arduous bond between tAK and substraight material
- 1% Sampling in manufacturing



Enzymatic Biological Indicator Strips



Luminometer – PR2A



PR2 A Reader

Manufactured for 20 Years.
Pharma Approved



Validatable Performance

IQ, OQ, PQ developed and easily
qualifiable to a light standard



Robust and Simple

Designed for constant use and
transportation.



Low capital and running cost

Designed to be low cost and inexpensive
to purchase and operate

ATHENA



Extensively Tested and Developed

Standard icon driven reading platform



Simple and intuitive

Easy to use with simple training



Safety built in

Positive and Negative controls before reading exposed strips



Rapid results

Quantifiable data delivered in 60 seconds per test strip.



Athena --- SIMULATION ---

Standard Cycle Enter Assay Result Name

Assay Finished.

Fractional Cycle Sample 1

Assay Details Show Log Reduction

No	Type	Timestamp	Value [RLU]	Log Reduction	Comment
1	Negative Control	14-Feb-2018 09:23:32 AM	2.27E+005		
2	Positive Control	14-Feb-2018 09:30:03 AM	1.00E+008		
3	Exposed Strip	14-Feb-2018 09:41:54 AM	7.47E+007	< 2.5	1 minute
4	Exposed Strip	14-Feb-2018 10:01:16 AM	2.47E+007	< 2.5	2 minutes
5	Exposed Strip	14-Feb-2018 10:07:58 AM	2.03E+007	< 2.5	3 minutes
6	Exposed Strip	14-Feb-2018 10:10:28 AM	5.46E+006	2.6	4 minutes
7	Exposed Strip	14-Feb-2018 10:17:34 AM	4.76E+006	3.0	5 minutes
8	Exposed Strip	14-Feb-2018 10:29:47 AM	4.22E+006	3.4	6 minutes
9	Exposed Strip	14-Feb-2018 10:35:45 AM	3.88E+006	3.7	7 minutes
10	Exposed Strip	14-Feb-2018 10:38:10 AM	3.17E+006	4.3	8 minutes
11	Exposed Strip	14-Feb-2018 10:40:37 AM	2.65E+006	4.9	9 minutes
12	Exposed Strip	14-Feb-2018 10:45:05 AM	1.82E+006	6.1	10 minutes
13	Exposed Strip	14-Feb-2018 11:07:58 AM	1.16E+006	7.5	11 minutes
14	Exposed Strip	14-Feb-2018 11:10:28 AM	1.00E+006	8.0	12 minutes
15	Exposed Strip	14-Feb-2018 11:12:58 AM	7.67E+005	8.8	13 minutes
16	Exposed Strip	14-Feb-2018 11:15:21 AM	6.12E+005	> 9.0	14 minutes
17	Exposed Strip	14-Feb-2018 11:42:50 AM	4.11E+005	> 9.0	15 minutes
18	Exposed Strip	14-Feb-2018 11:46:04 AM	3.87E+005	> 9.0	

Software Reporting



Un-editable Data Files

Once the data is delivered it cannot be edited



Content Rich Reporting

More data recorded with minimal user input



Safety built in

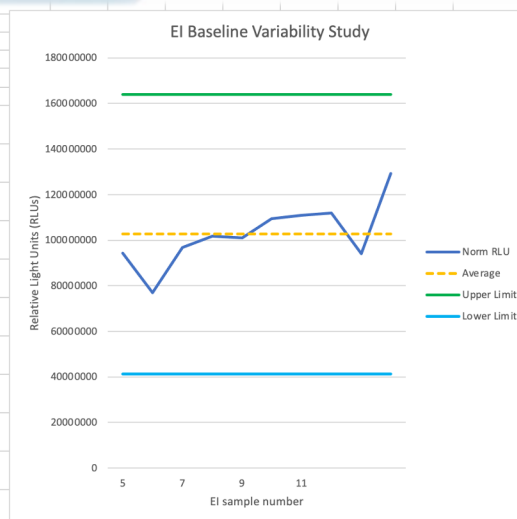
Positive and Negative controls before reading exposed strips



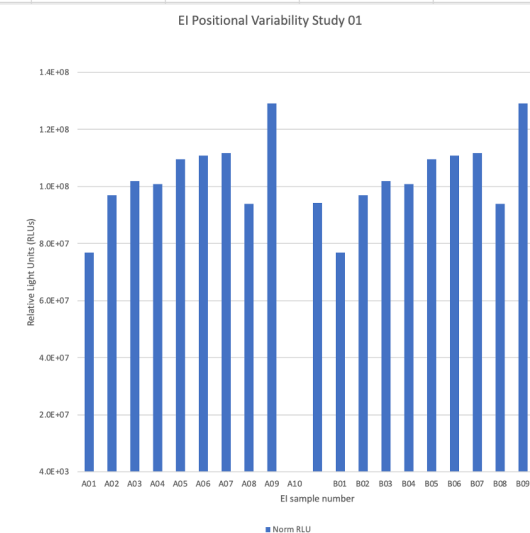
Software Reporting



	Average	Std Dev S	CV%	MAX	MIN	15%	-15%		
	1.03E+08	13935583.69	13.578	1.29E+08	7.69E+07	1.18E+08	8.72E+07		
Sample	Name	Date/Time	RLU	Norm RLU	Mean	MAX	Min	Lower Limit	Upper Limit
1	Negative Control	11/27/2020 6:03:12 PM	124635	0				80000	340000
2	Positive Control	11/27/2020 6:09:32 PM	94473492	94348857	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
3	Exposed Strip	11/27/2020 6:14:55 PM	77056279 < 2.5	76931644	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
4	Exposed Strip	11/27/2020 6:22:03 PM	97010859 < 2.5	96886224	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
5	Exposed Strip	11/27/2020 6:24:02 PM	101970969 < 2.5	101846334	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
6	Exposed Strip	11/27/2020 6:26:05 PM	101064623 < 2.5	100939988	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
7	Exposed Strip	11/27/2020 6:28:01 PM	109622982 < 2.5	109498347	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
8	Exposed Strip	11/27/2020 6:29:43 PM	111022601 < 2.5	110897966	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
9	Exposed Strip	11/27/2020 6:31:23 PM	111910664 < 2.5	111786029	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
10	Exposed Strip	11/27/2020 6:32:57 PM	94071531 < 2.5	93946896	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08
11	Exposed Strip	11/27/2020 6:34:42 PM	129355345 < 2.5	129230710	1.03E+08	1.18E+08	8.72E+07	4.13E+07	1.64E+08



	Average	Std Dev S	CV%	MAX	MIN
LOC A	9.32E+07	35468638.01	38.058	1.29E+08	0.00E+00
LOC B	1.04E+08	14455031.51	13.959	1.29E+08	7.69E+07
Sample	Name	Date/Time	RLU	Norm RLU	
1	Negative Control	11/27/2020 6:03:12 PM	124635	0.00E+00	
2	Positive Control	11/27/2020 6:09:32 PM	94473492	9.43E+07	
A01	3 Exposed Strip	11/27/2020 6:14:55 PM	77056279 < 2.5	7.69E+07	
A02	4 Exposed Strip	11/27/2020 6:22:03 PM	97010859 < 2.5	9.69E+07	
A03	5 Exposed Strip	11/27/2020 6:24:02 PM	101970969 < 2.5	1.02E+08	
A04	6 Exposed Strip	11/27/2020 6:26:05 PM	101064623 < 2.5	1.01E+08	
A05	7 Exposed Strip	11/27/2020 6:28:01 PM	109622982 < 2.5	1.09E+08	
A06	8 Exposed Strip	11/27/2020 6:29:43 PM	111022601 < 2.5	1.11E+08	
A07	9 Exposed Strip	11/27/2020 6:31:23 PM	111910664 < 2.5	1.12E+08	
A08	10 Exposed Strip	11/27/2020 6:32:57 PM	94071531 < 2.5	9.39E+07	
A09	11 Exposed Strip	11/27/2020 6:34:42 PM	129355345 < 2.5	1.29E+08	
A10	1 Negative Control	11/27/2020 6:03:12 PM	124635	0.00E+00	
	2 Positive Control	11/27/2020 6:09:32 PM	94473492	9.43E+07	
B01	3 Exposed Strip	11/27/2020 6:14:55 PM	77056279 < 2.5	7.69E+07	
B02	4 Exposed Strip	11/27/2020 6:22:03 PM	97010859 < 2.5	9.69E+07	
B03	5 Exposed Strip	11/27/2020 6:24:02 PM	101970969 < 2.5	1.02E+08	
B04	6 Exposed Strip	11/27/2020 6:26:05 PM	101064623 < 2.5	1.01E+08	
B05	7 Exposed Strip	11/27/2020 6:28:01 PM	109622982 < 2.5	1.09E+08	
B06	8 Exposed Strip	11/27/2020 6:29:43 PM	111022601 < 2.5	1.11E+08	
B07	9 Exposed Strip	11/27/2020 6:31:23 PM	111910664 < 2.5	1.12E+08	
B08	10 Exposed Strip	11/27/2020 6:32:57 PM	94071531 < 2.5	9.39E+07	
B09	11 Exposed Strip	11/27/2020 6:34:42 PM	129355345 < 2.5	1.29E+08	



Software Reporting



FRM053_A - PR2A Performance Qualification Data Analysis Template

Initial 5 reps LB9517 and Blank Tube	
Sample	RLU/s
LB9517 - 1	824541
Blank - 1	2737
LB9517 - 2	799606
Blank - 2	2737
LB9517 - 3	816229
Blank - 3	3649
LB9517 - 4	811611
Blank - 4	1825
LB9517 - 5	829158
Blank - 5	3649
Average LB9517	816229
Average Blank Tube	2919.4

10 x Reps for Accuracy (same chamber)	
824541	
799606	
816229	
811611	
829158	%CV
799606	1.42
808841	Deviation %
795912	1.15
815306	STDEV
823617	11497.146
Mean	AVDEV
812442.7	9327.5

10 x Reps for Precision (5 in each chamber)	
799606	
808841	
795912	
815306	
823617	%CV
825714	2.09
857653	Deviation %
822064	1.44
817500	STDEV
825714	17136.1455
Mean	AVDEV
819192.7	11759.7

	Injector 1	Injector 2
Injector Results 50 µl	48.9	48.9
	50.5	49.7
	49	49.9
	50.6	49.7
	50	49.7
	Average	49.8
STD	0.809320703	0.389871774
CV	1.625141974	0.786348878
Injector Results 100 µl	103	99.6
	101.3	99
	100.1	99.1
	100.2	99.4
	100	97.6
	Average	100.92
STD	1.275539102	0.78612976
CV	1.263911119	0.794552012
Injector Results 150 µl	152.6	141
	151.9	151
	151.7	148.4
	152	151.3
	151.9	147.7
	Average	152.02
STD	0.342052628	4.155357987
CV	0.225005017	2.809952656

Raw Results Accuracy

824541	799606
2737	2737
799606	808841
2737	5474
816229	795912
3649	3649
811611	815306
1825	912
829158	823617
3649	3649
799606	1846
2737	825714
808841	2770
5474	857653
795912	1846
3649	822064
815306	6463
912	817500
823617	2770
3649	825714
	1846

10 X Reps for Precision (5 in each chamber)

799606
2737
808841
5474
795912
3649
815306
912
823617
1846
825714
2770
857653
1846
822064
6463
817500
2770
825714
1846

RLU Factor	1282.738
LB9517 Target Value	777620
New RLU Factor	1222.0623



EI Variation

Unexposed strips - Order vs RLU



QC Test process

- **Positive: 95% of N=60 unexposed strips (95-100% Acceptable)**
- **Mean RLU = 9.09E+07 (Acceptable Range 6.00E+07 – 1.64E+08)**
- **%CV = 9.90% (Acceptable Range = < 15%)**

EL Variation

Variability: Cumulative effect not just tAK variability



**Reader Variation = <2% @
1.5% CV from blank tube /
luminometer read variance
(PQ)**



**Test Strip Variation = < 10%
(6.25% if subtracting Reader
and Reagent variation)**



**Reagent Variation
≤5% @ 3.65% (2.15 % CV
if subtracting above
reader CV)**

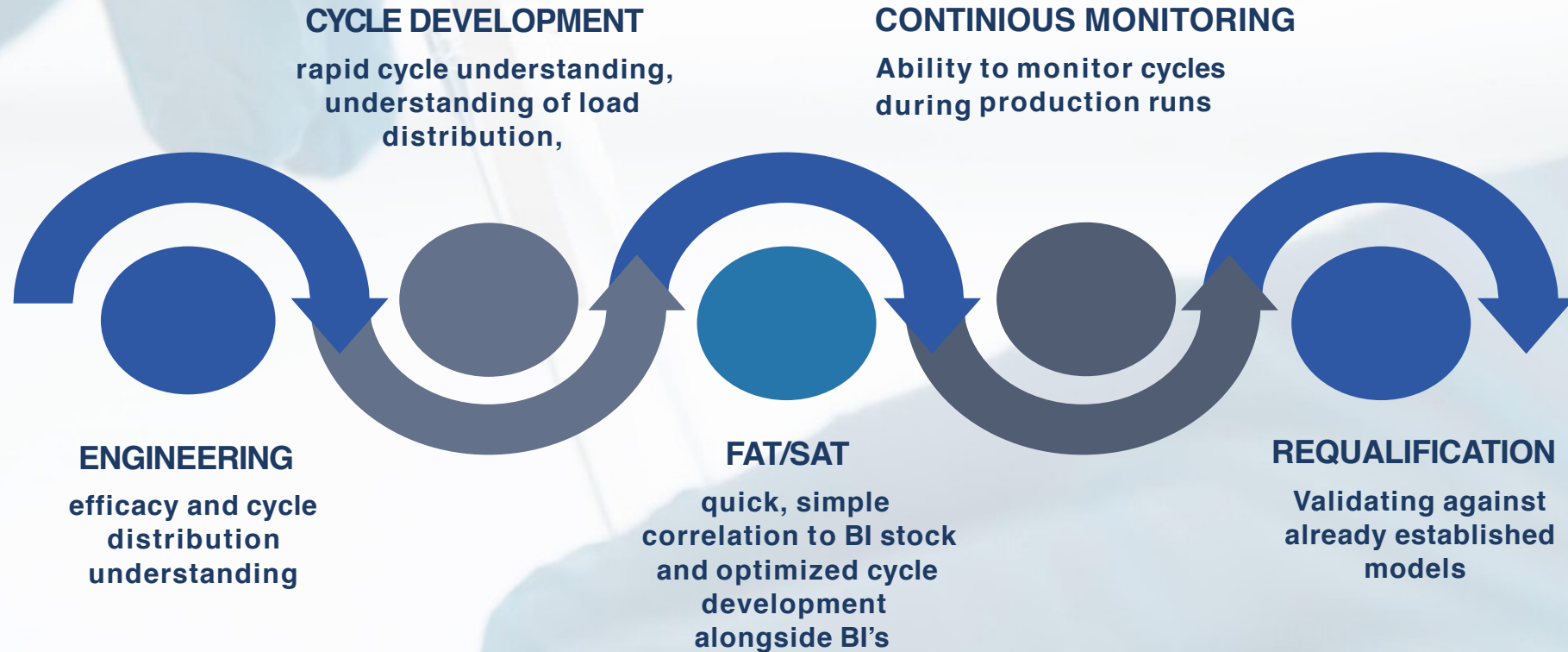
This equals to the 9.9% total (<15%)



Application and Benefits

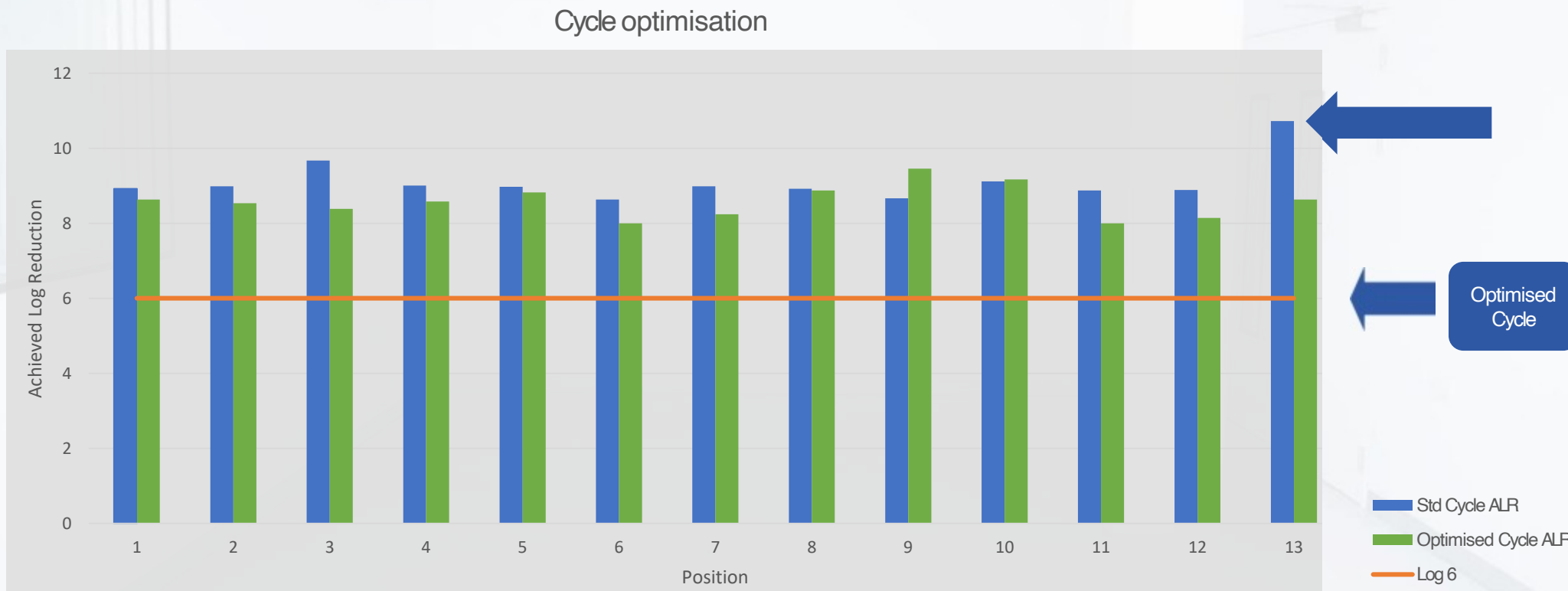


When can EI's be applied?



Cycle optimisation

Customer cycle development to improve cycle efficiency whilst ensuring log6 reduction was achieved



CYCLE TIME REDUCED BY 50%

Positional/distribution studies

tAK EI VPHP inactivation isolator; full cycle: Effect of position

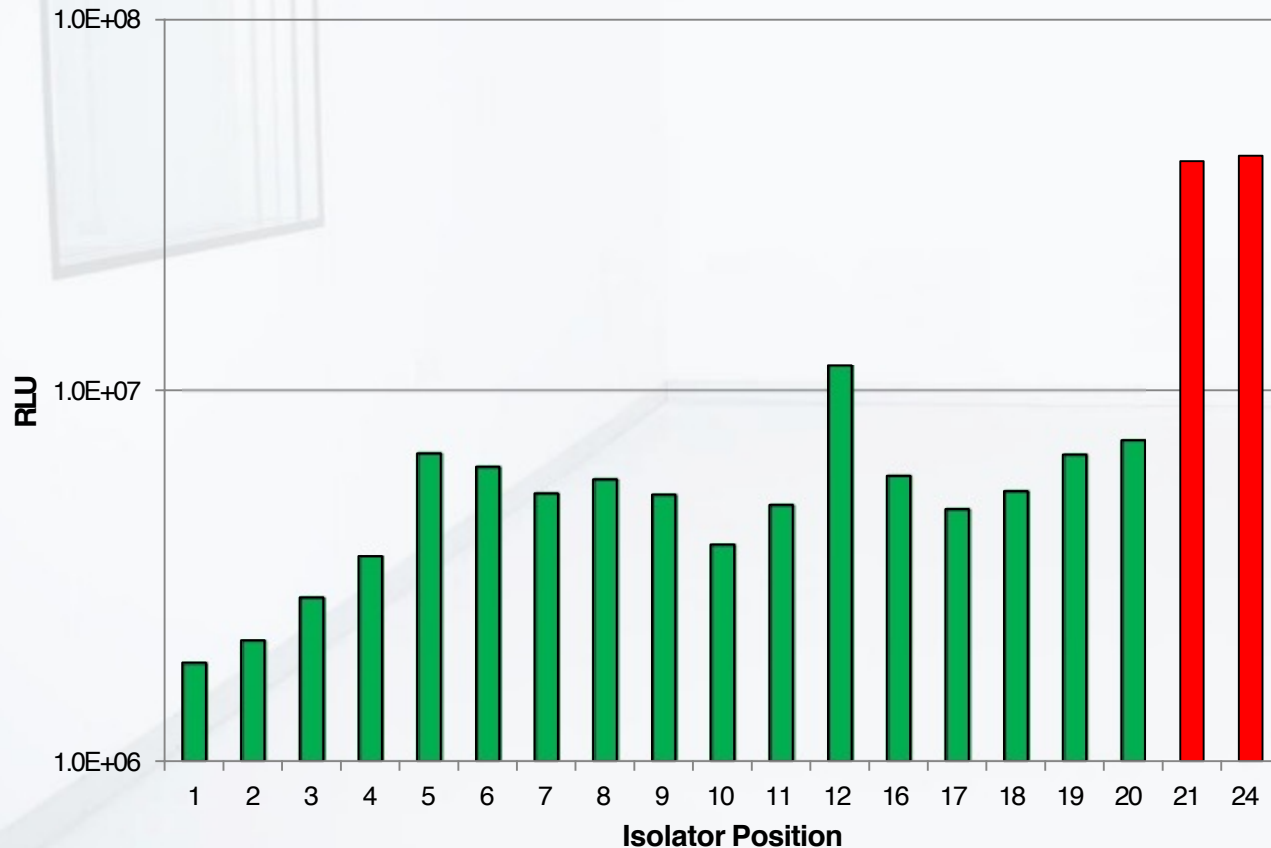


Figure shows EI RLU values at each position. Green bars signify no BI growth, red bars BI growth

BI growth was observed at positions 21 and 24. These position are isokinetic sampling probes and known to be challenging.

No BI growth at the position with the next highest RLU (position 12- under the removable floor of the isolator.

Financial Benefits

Faster

- Improved TAT
- Earlier batch release

Smarter

- Increased Capacity
- Quantifiable data on cycle efficiency

Safer

- Assured Cycle efficacy
- Real-Time Data

Cost Effective

- Save £\$
- Significant time savings

5 year cost savings per region*



*Calculated for a 200m area, comparison is for BI's in triplicate with CI's vs Single BI's & Single EI's and no CI's

Enzymatic Decontamination Validation Delivers



Knowledge

EI's deliver new levels of data simply and effectively.



Continuous Validation

Engineered to deliver validation control and qualification on every read.



Process Understanding

EI's facilitate spot checks and continuous cycle qualification



Preventing Failure

Removing RUN TO FAIL from decontamination process.



Regulatory Compliance

Substantially more robust and reproducible challenge



60 Second Results

7 day incubation reduced to 60 second read. **Saving 1000's of hours per year**



Large financial gains

EI technology will deliver **multimillion dollar savings every year**



Improving

EI technology will replace BI's because on every level it outperforms.



PROTAK SCIENTIFIC

THANK YOU FOR LISTENING

Paul Liu

Softbio Health Inc.

晁丞健康股份有限公司



PROTAK SCIENTIFIC

EI Q&A

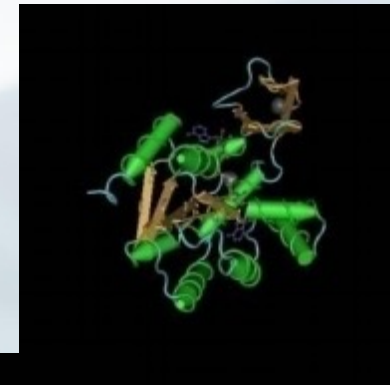
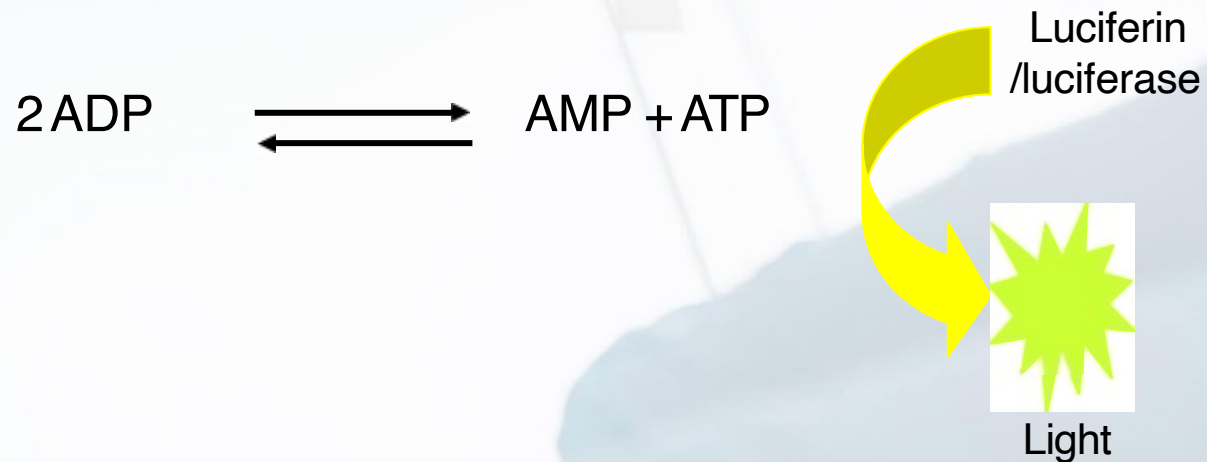
Softbio Health Inc.

晁丞健康股份有限公司

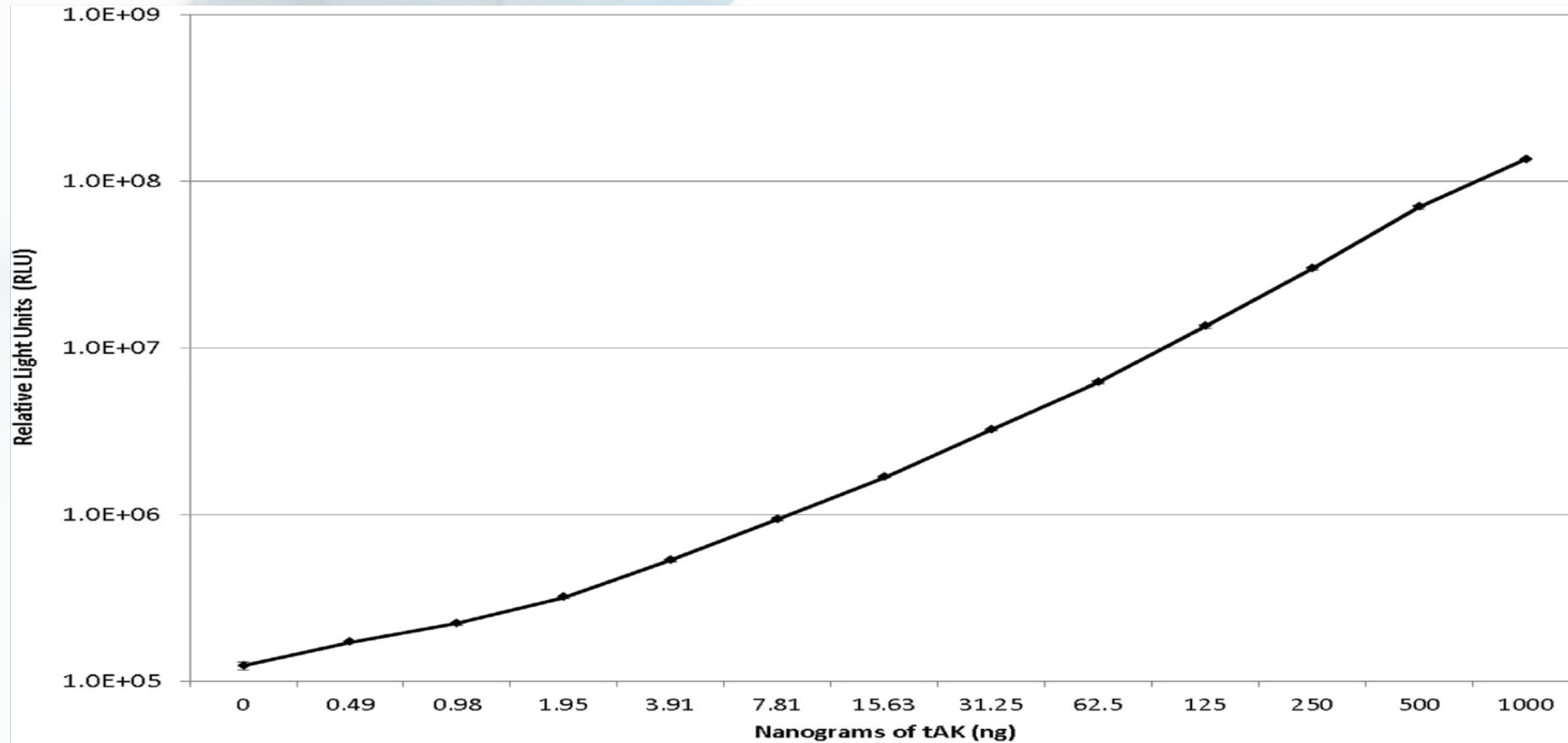


Rapid detection systems used for decontamination monitoring

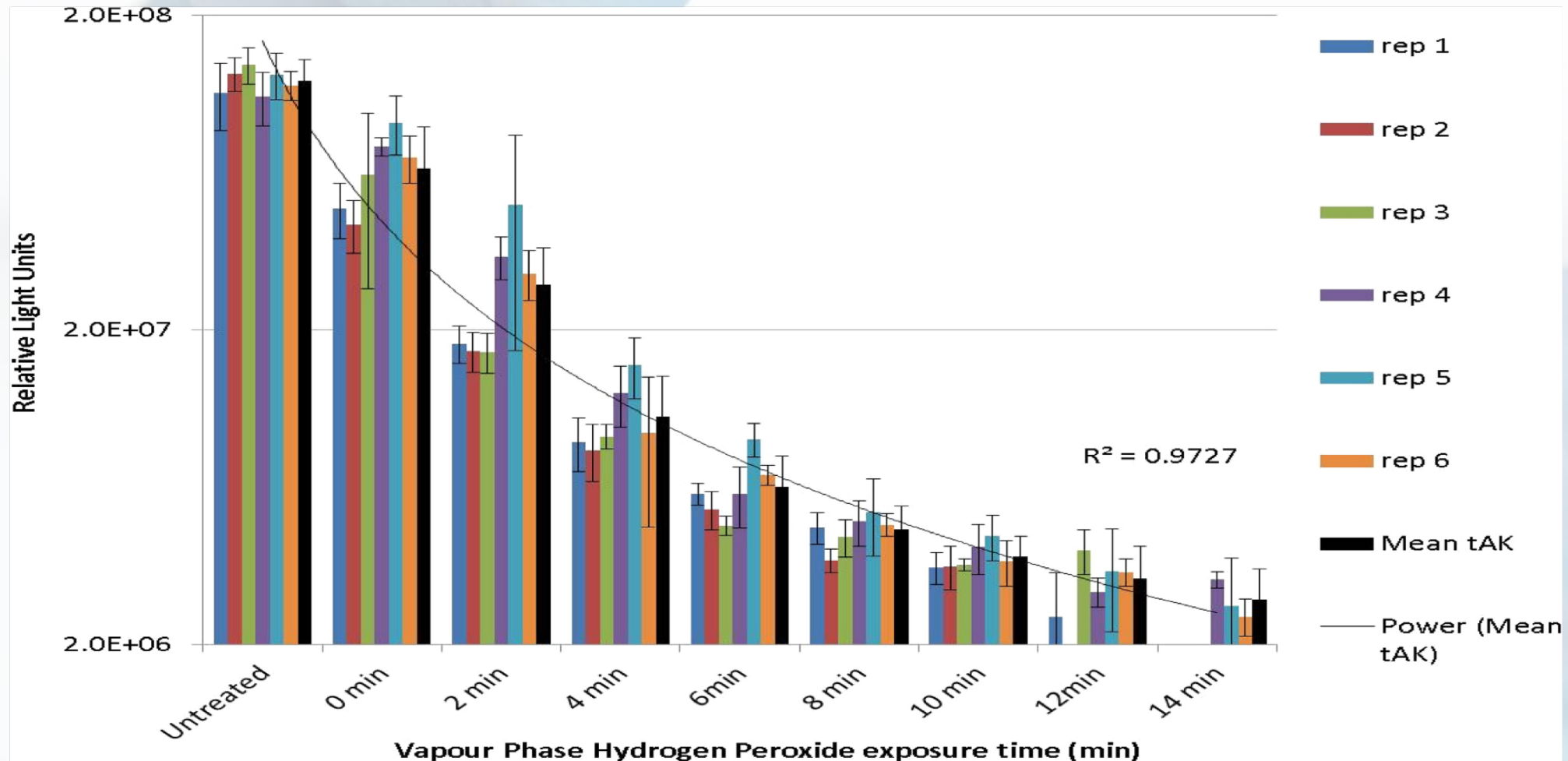
- Thermostable adenylate kinases (tAKs)
- Isolated from thermophilic bacteria in volcanic springs; *Sulpholobus acidocaldarius*



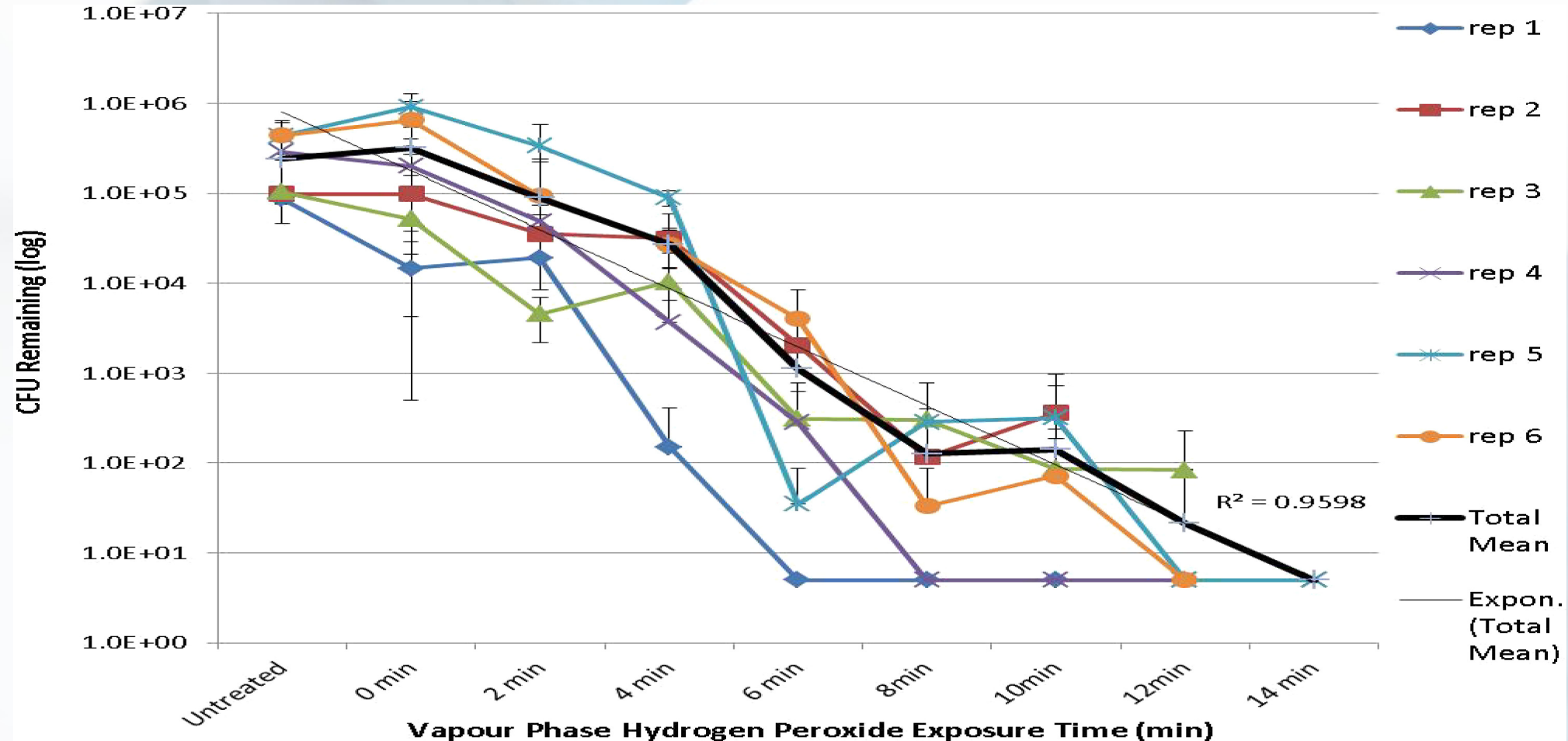
tAK Standard Curve



Residual tAK activity expressed as relative light units (RLU) following hydrogen peroxide exposure in a flexible film isolator



Time course of inactivation of *Geobacillus stearothermophilus* spore discs during replicate hydrogen peroxide decontamination processes



Comparing BI and EI inactivation profiles

