A POC Test for Detection of Resistance to Neuraminidase Inhibitors

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Topics

- Homogeneous Biochemiluminescence Assays (HBA) and Its Use for Detection of Influenza Viral Neuraminidase (NA)
- QFlu Combo Test for POC Use in Detection of Resistance to NA Inhibitors
- qCPO Assay for Detection of Superbugs (Carbapenemase-Producing Organisms)

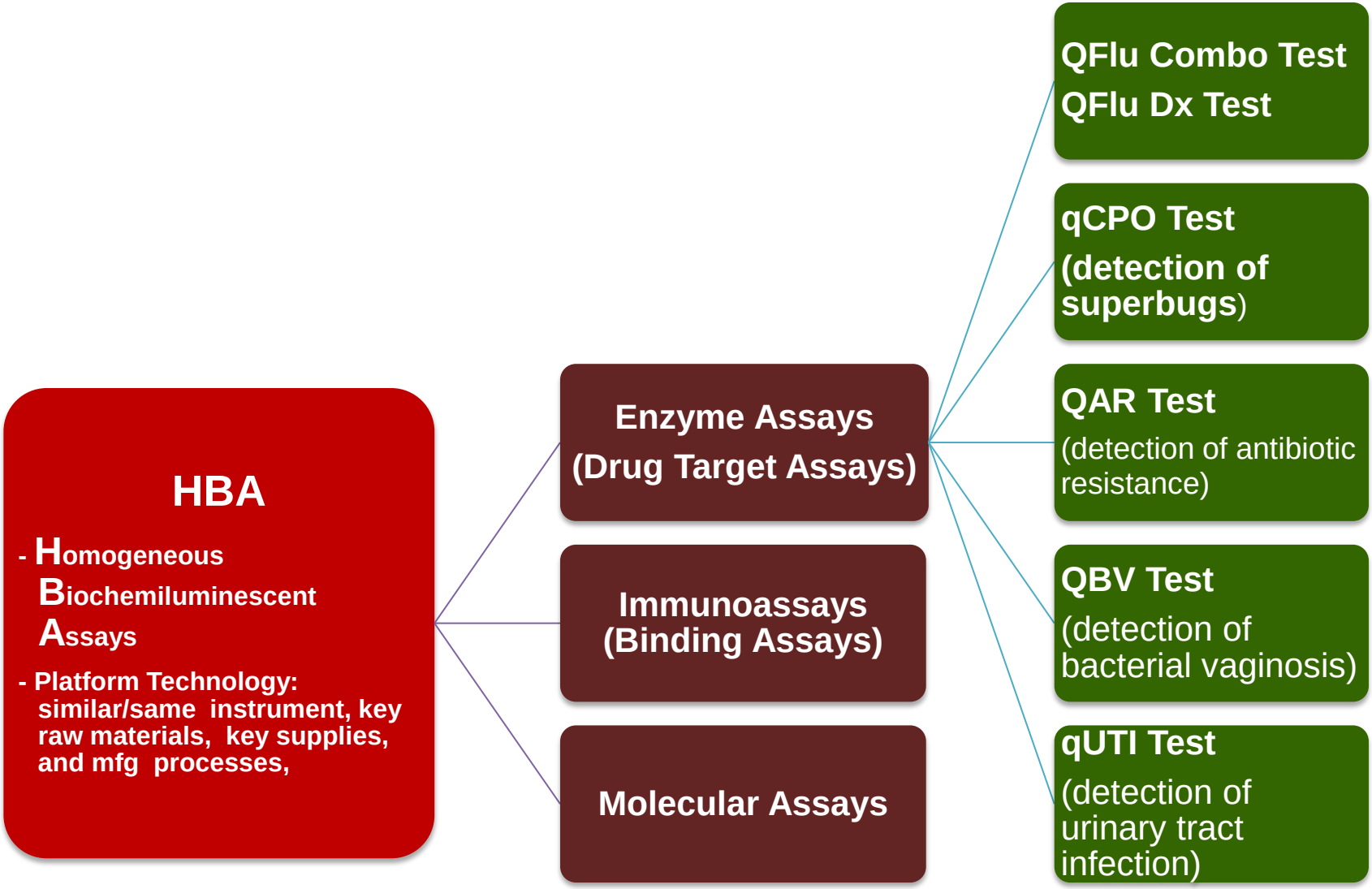
Desired Attributes of a POC Test

- ❑ **Suitable for Physician's Office Use (POC)**
- ❑ **Easy-to-use (7th grade education level)**
- ❑ **Super Performance Characteristics**
  - **Rapid (< 30 min)**
  - **Sensitive and Specific**
  - **Qualitative and Quantitative**
- ❑ **Inexpensive**

Homogeneous Biochemilumincescent Assays (HBA)

- Non washing
- Based on firefly biochemiluminescence
- A platform technology
- Suitable for POC use

HBA-based Assays

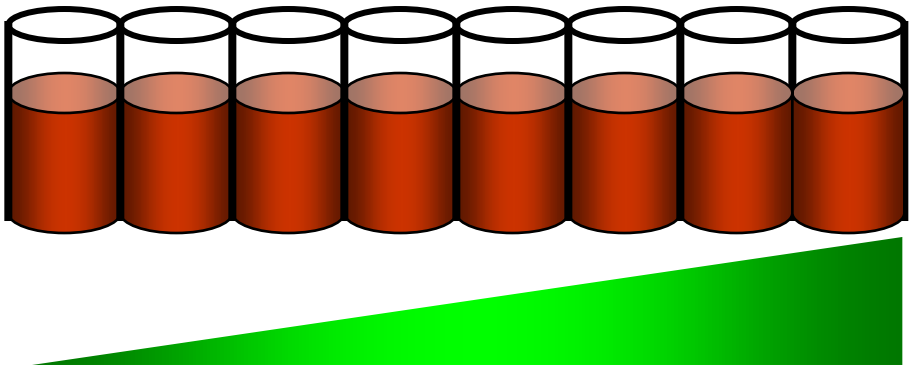


Current Methods for Detection of NAI Susceptibility

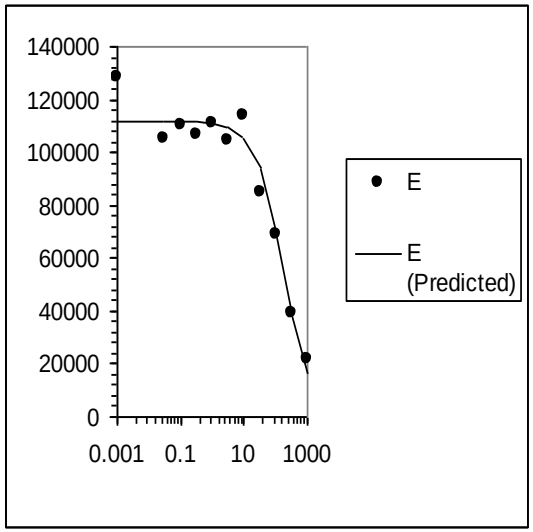
- **Cell Culture Based Inhibition Assays**
- **Molecular Assays for Detection of Genetic Mutations**
  - Sequencing Assays
  - Nucleic Acid Amplification Assays (e.g. PCR)
- **NA Inhibition Assays for Estimation of IC_{50}**

Not suitable for POC use.

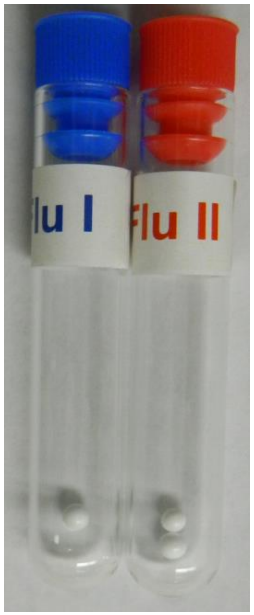
IC_{50} Assay



Increasing NA Inhibitor Concentration



Two Reaction Assay

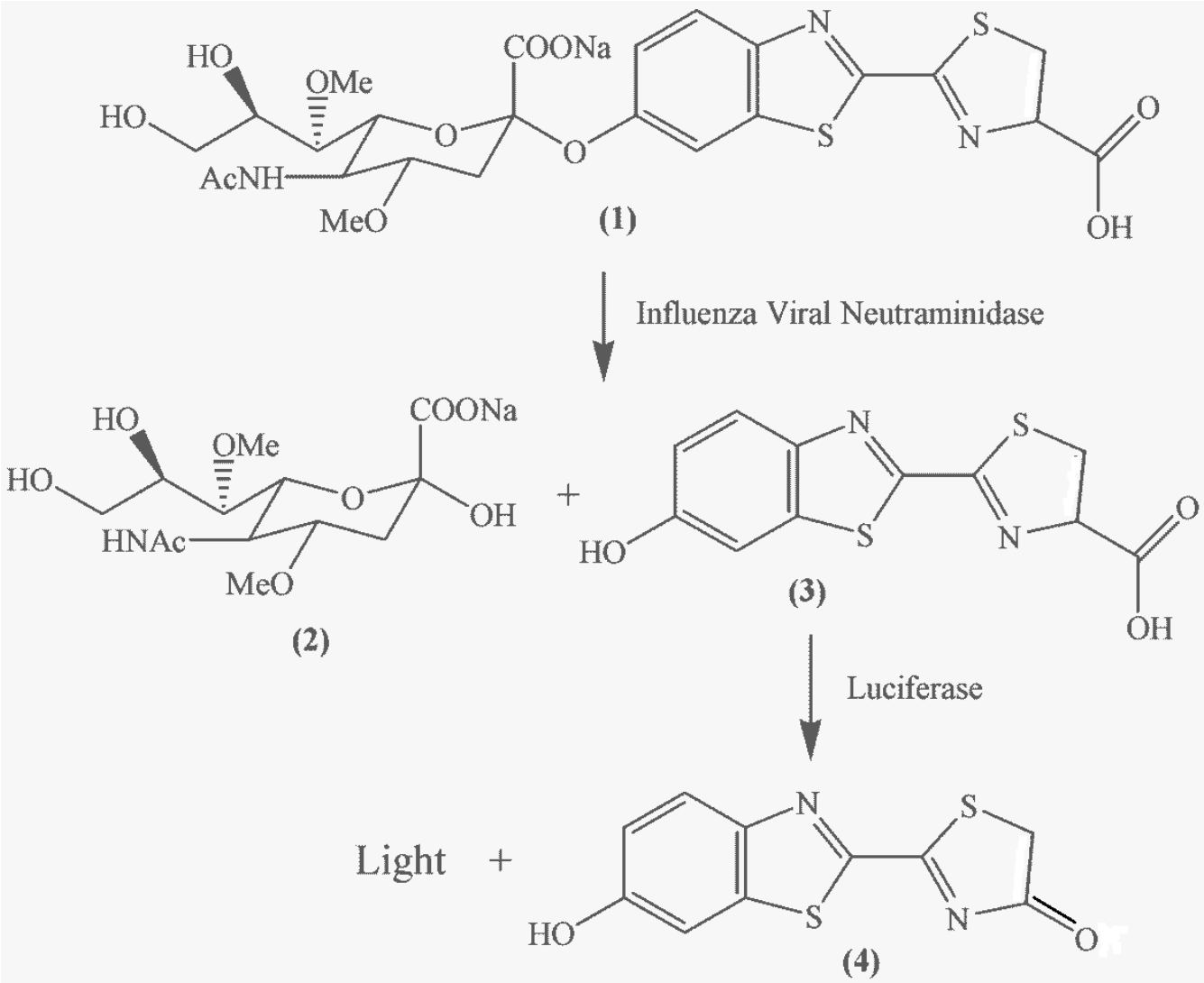


R-Factor = $\frac{\text{10 x Reagent II Signal}}{\text{Reagent I Signal}}$

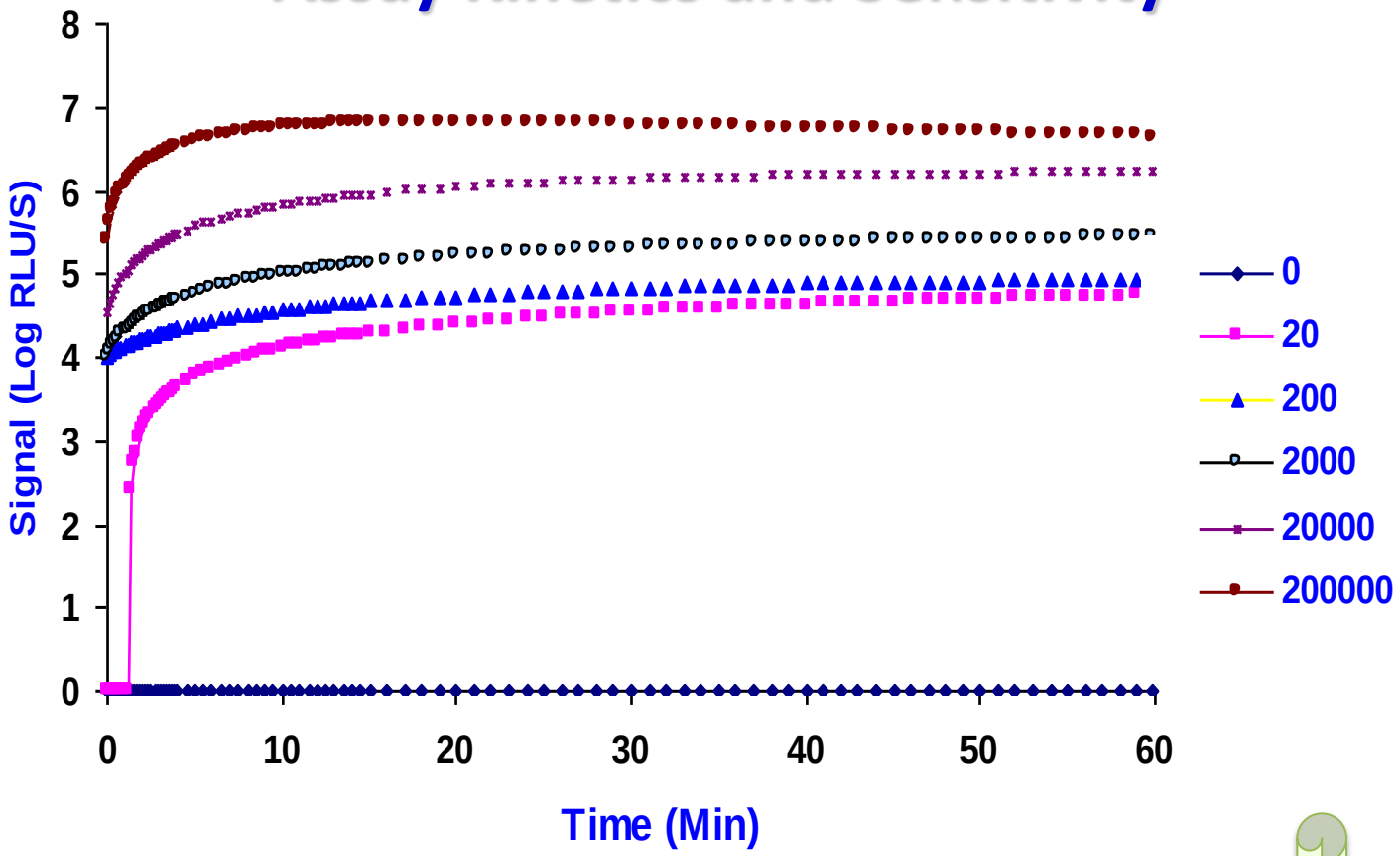
Assay Requirements:

- Easy to use
- Quantitative
- Reproducible

Biochemical Reactions in QFlu Assays



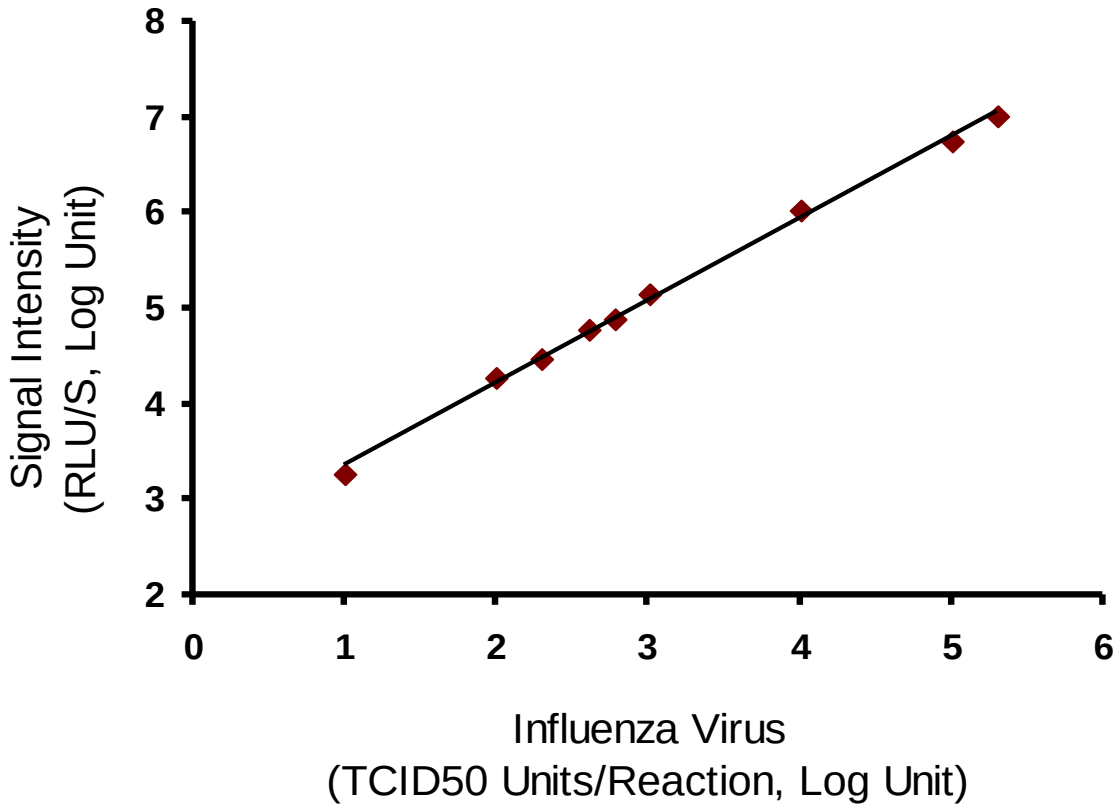
Assay Kinetics and Sensitivity



Reaction Kinetics

Increasing amounts in $TCID_{50}$ Units of flu virus were added to the reaction mix. The signal was measured over a period of 60 minutes. The assay was sensitive and exhibited stable signal for at least 60 minutes.

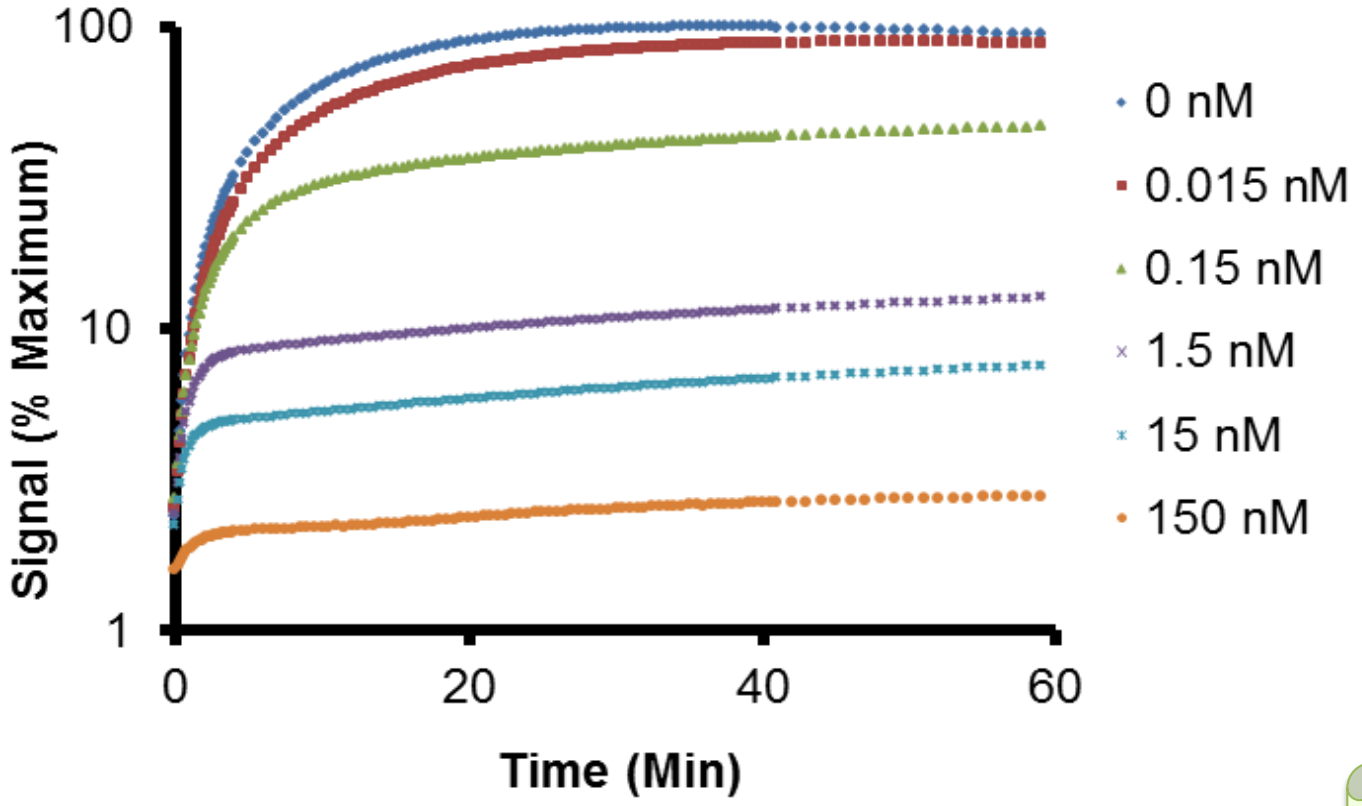
Assay Linear Range



Linearity

The QFlu assay has great linear range and linearity, suitable for quantitation and drug resistance detection.

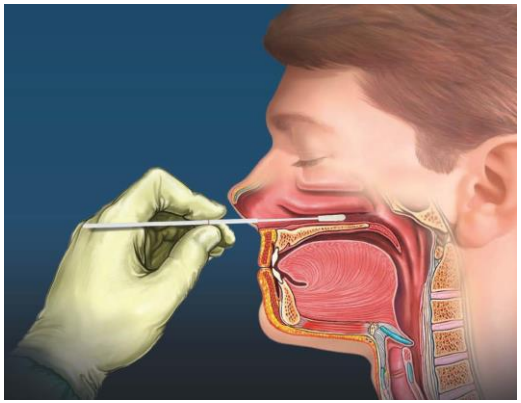
Used as an Inhibition Assay



Inhibition Kinetics

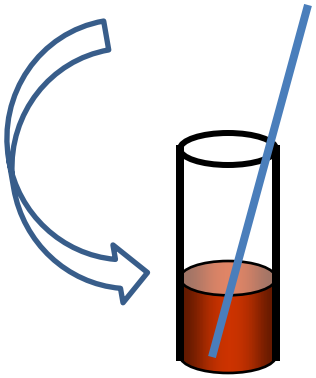
Increasing amounts in Tamiflu (oseltamivir) was added to a reaction mix containing flu virus. The signal was measured over a period of 60 minutes. When compared to the control reaction without Tamiflu, inhibition was immediately evident and stabilizes within 15 minutes.

QFlu Combo Test for POC Use



1. Sample Collection

- ❑ Collect a NG Swab
- ❑ Elute the sample

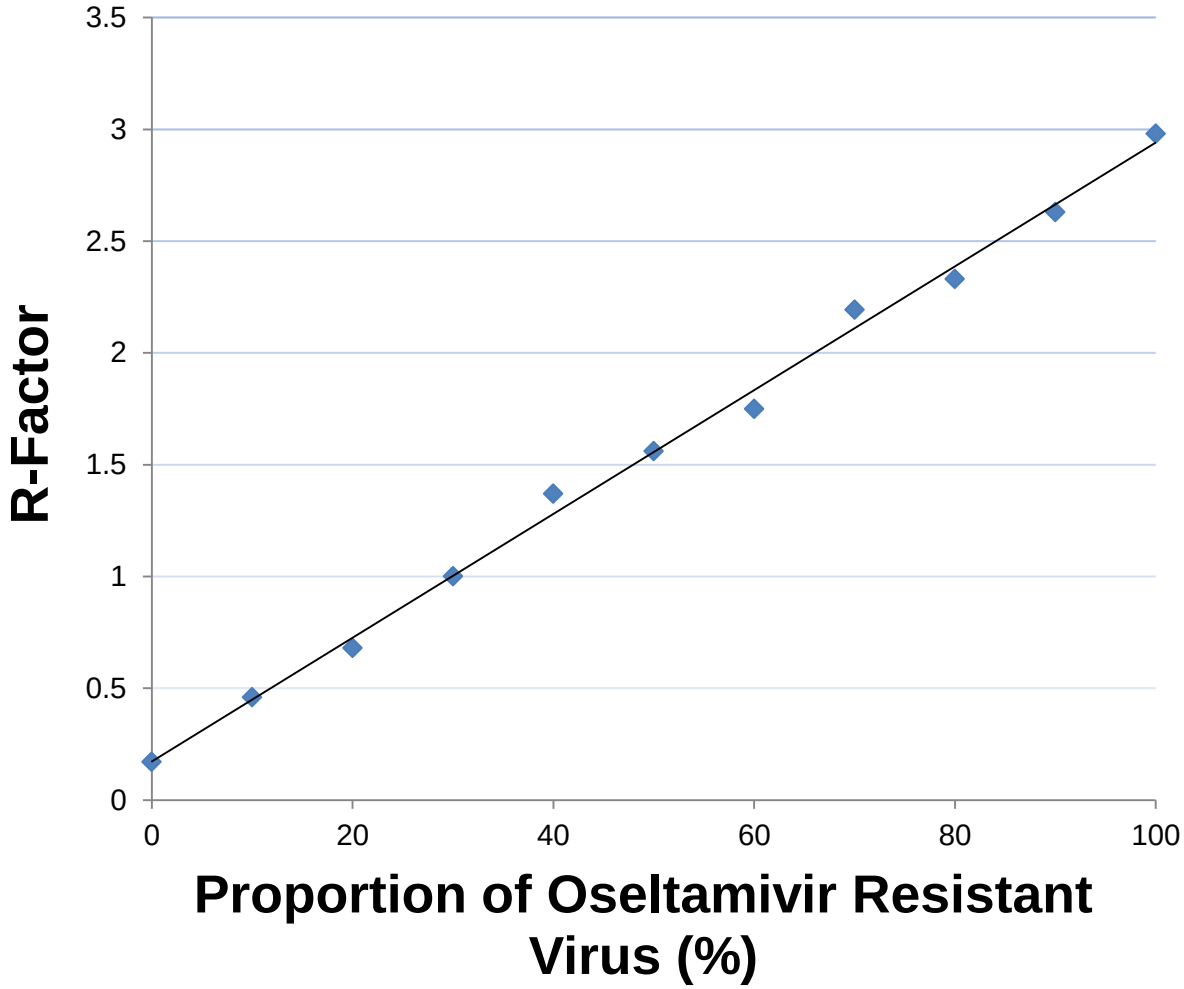


3. Signal Measurement and Result Interpretation

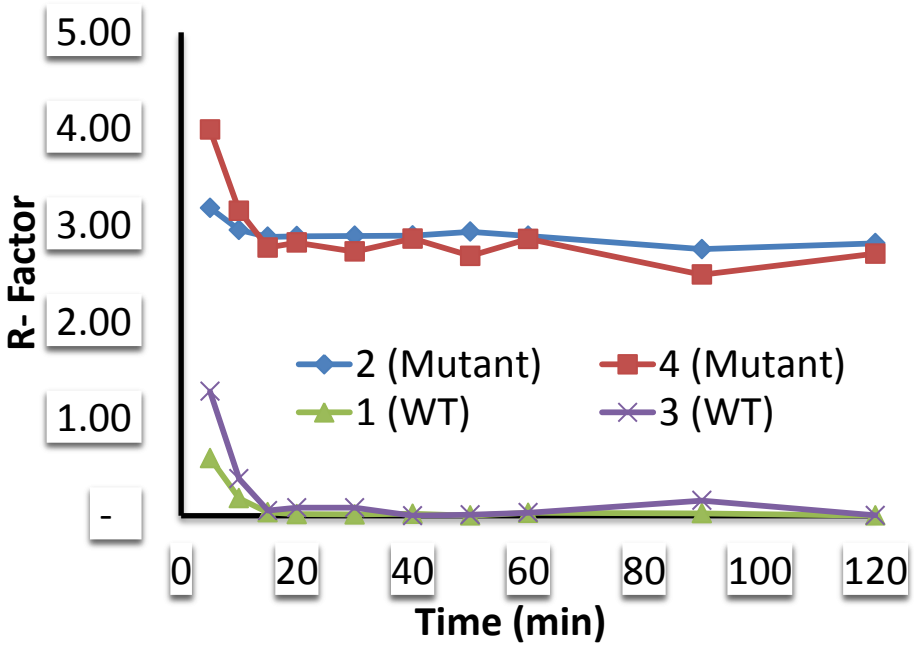
2. Reaction

- ❑ Sample Addition to Reagents I & II;
- ❑ Incubation for 15 min at Room Temp

Relationship Between R-Factor Values and NA Inhibitor "Susceptibility"



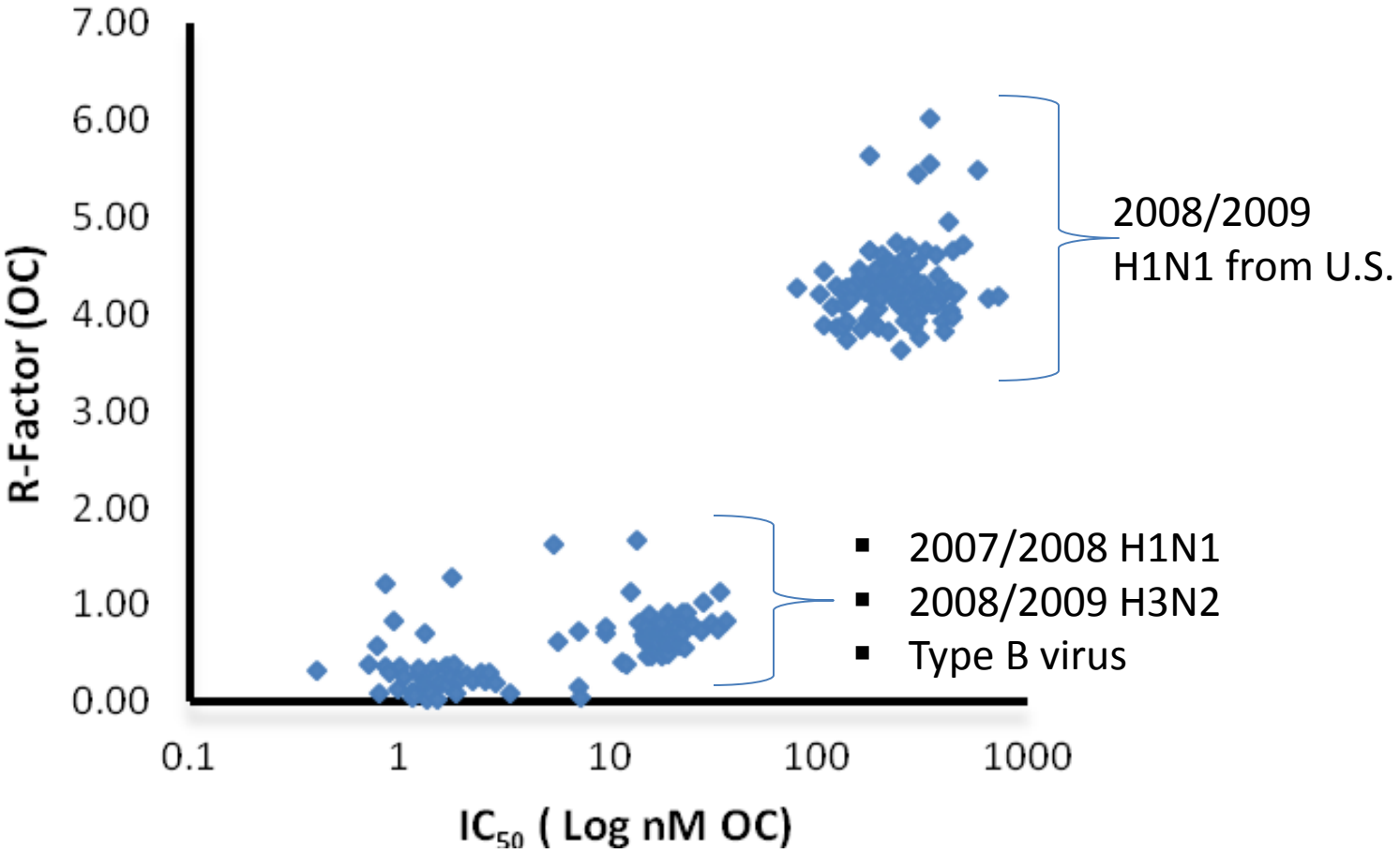
Inhibition Kinetics (QFlu Combo Test)



Panel Member	Virus Strain	Tamiflu Resistance	Virus Level
1	A/CA/07/2009	S	H
2	A/NC/39/2009	R	H
3	A/CA/07/2009	S	M
4	A/NC/39/2009	R	M

Signal Ratios Were Drastically Different between Tamiflu Susceptible and Resistant (mutant) Flu Virus as Indicated by R-Factors.

Detection of Tamiflu Resistance



QFlu Test Kits (CE-Marked)

QFlu Dx Test

QFlu Combo Test

Superbug linked to 2 deaths at UCLA hospital; 179 potentially exposed

UCLA's Ronald Reagan Medical Center has begun notifying patients that they may have been exposed to a deadly superbug. (Francine Orr / Los Angeles Times)

What Are Superbugs?
(Carbapenemase Producing Organisms – CPO)

- Superbugs are bacterial species that produce carbapenemase and are hence resistant to carbapenems (antibiotics);
- Carbapenems are used as the last-resort antibiotics;
- Bacterial species resistant to carbapenems are often resistant to other antibiotics, hence “superbugs”;

Resistance to Carbapenems

- Enabled by carbapenemases, which are
  - a type of β lactamases that can degrade carbapenems;
  - encoded by mobile plasmids, which can be easily transmitted between species
- Infection rates in common Enterobacteriaceae (in U.S.):
  - 2001: 1.2%
  - 2011: 4.2%

Similarity between qCPO Assay and Other Assays

	QFlu Combo Test	QAR	qCPO
Intended Use	Detection of NAI resistance	Detection of β-lactamase inhibitor resistance	Detection of "superbugs"
# of Reagents	2	2	2
R-Factor	Yes	Yes	Yes
Target Enzyme	Flu Viral NA	Beta Lactamase	Carbapenemase
Target Inhibitor	NA Inhibitors	β-Lactamase Inhibitor	Carbapenems
Inhibitor Example	Oseltamivir	Clavulanate	Imipenem

CPO: Carbapenemase Producing Organisms - Superbugs

Detection of Recombinant β Lactamases

β Lactamase			S/N	R-Factor	
Class	Name	Carbapenemase?		Clavulanate (QAR Assay)	Imipenem (qCPO Assay)
1	p99	No	48	10.00	0.31
2a	SHV2	No	44	0.28	2.47
2b	TEM2	Yes / No	45	8.83	9.56
2c	KPC2	Yes	42	1.11	5.60
3	NDM-2	Yes	22	5.89	6.18

R-Factor cutoff : 2.50

Detection of Fresh Clinical Isolates – QAR Assay

No.	Sample ID	Bacterial Sp.	Reagent I S/CO	R-Factor**	Beta lactamase	CA Resistance
1	1845	*Klebsiella pneumoniae*	9.27	0.07	Yes	No
2	1847	*Klebsiella pneumoniae*	8.58	0.15	Yes	No
3	1820	*Pseudomonas aeruginosa*	1.26	3.67	Yes	Yes
4	1765	*Acinetobacter baumannii*	0.33	N/A	No	N/A
5	B612	*E coli*	22.83	0.27	Yes	No
6	B616	*E coli*	16.62	0.13	Yes	No
7	1789	*Pseudomonas aeruginosa*	16.36	1.14	Yes	No
8	1800	*Pseudomonas aeruginosa*	9.79	11.13	Yes	Yes
9	B654	*Pseudomonas aeruginosa*	0.41	N/A	No	
10	2046	*Klebsiella pneumoniae*	0.11	N/A	No	
11	2065	*Acinetobacter baumannii*	0.14	N/A	No	
12	1974	*Acinetobacter baumannii*	0.11	N/A	No	
13	1962-2	*Pseudomonas aeruginosa*	5.29	15.47	Yes	Yes
14	1963	*Pseudomonas aeruginosa*	2.42	19.95	Yes	Yes
15	B678	*E coli*	0.72	N/A	No	
16	2017	*Acinetobacter baumannii*	0.20	N/A	No	
17	2015	*Acinetobacter baumannii*	7.24	1.74	Yes	No
18	2022-1	*Acinetobacter baumannii*	1.10	1.31	Yes	No
19	1959	*Klebsiella pneumoniae*	0.08	N/A	No	
20	B650	*Klebsiella pneumoniae*	7.89	0.11	Yes	No
21	B671	*Klebsiella pneumoniae*	4.15	4.12	Yes	Yes
22	1962-1	*Klebsiella pneumoniae*	0.42	N/A	No	
23	1978	*Acinetobacter baumannii*	0.67	N/A	No	

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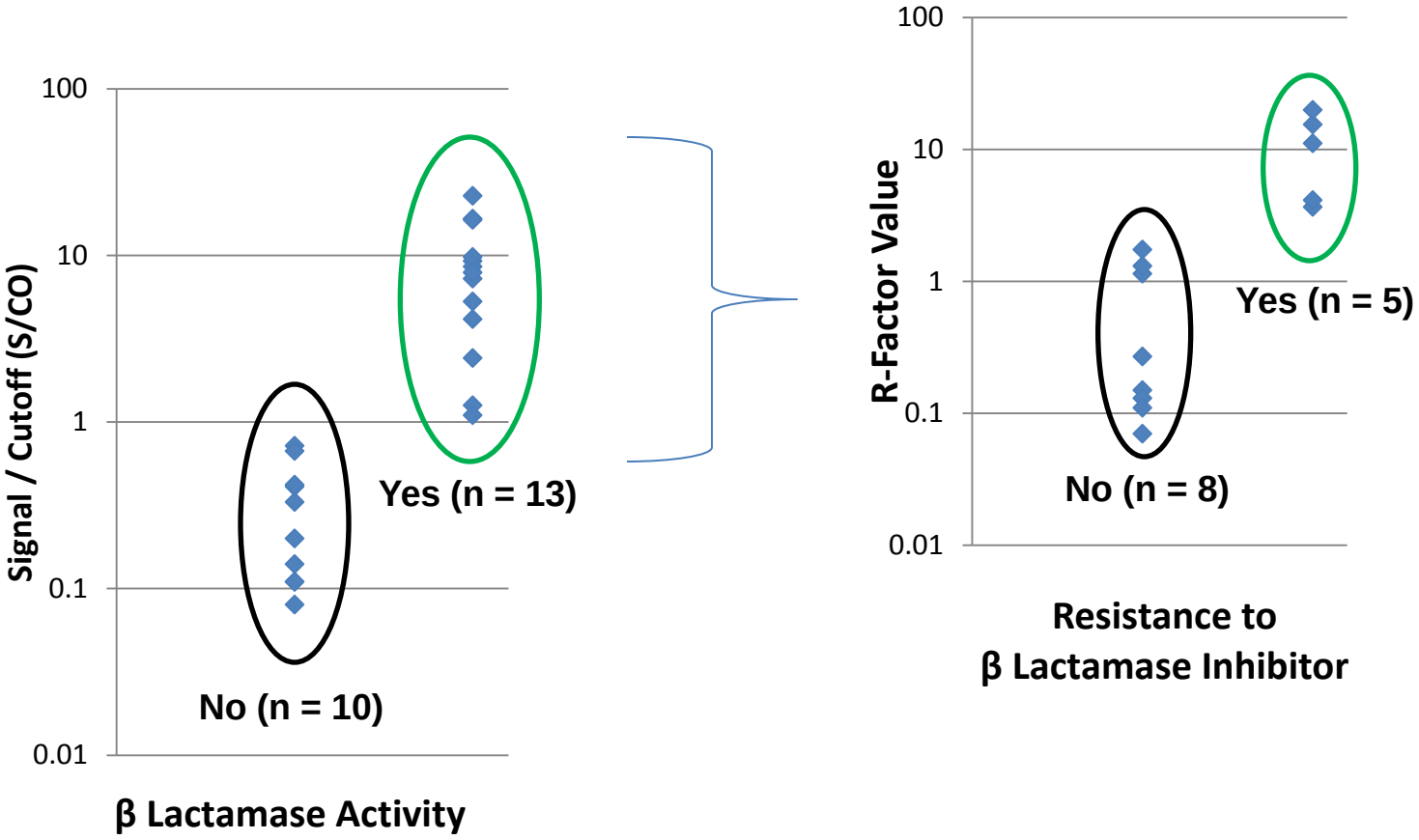
Summary – QAR Assay



Summary

- HBA-based assays are easy to use, sensitive and quantitative;
- HBA-based assays in two reactions format simplifies drug resistance detection and could be used in POC settings;
- Since enzymes are targets for many therapeutic drugs, this approach can be used for detection of resistance to a large number of drugs.

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Collaboration Partners

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