ViroSpot™ Assay for 24-hour phenotypic influenza virus titration

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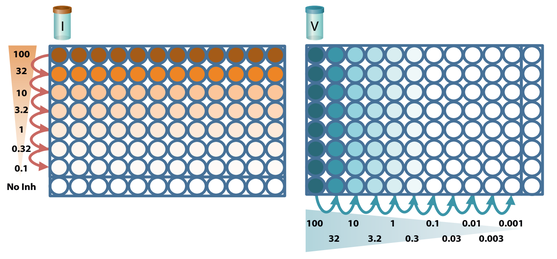
INTRODUCTION

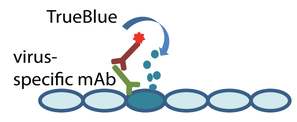
- **To obtain virus isolates for phenotypic analysis, clinical specimens are generally cultured and titrated in absence of inhibitors prior to further characterization.**
- **Proportions of virus variants may change during culture, and minority species with reduced sensitivity may remain undetected.**
- **Here we developed sensitive detection technology for direct phenotypic characterization of virus in samples containing between 1E2 and 1E7 plaque forming unit (PFU)/mL:**
  - Within 24h after inoculation, without prior virus culture or titration
  - Facilitating isolation of clonal progeny virus populations for further phenotypic and genotypic analyses.
  - Automated imaging ensures objective data and traceability of infected cell patterns which formed in presence and absence of antiviral substances.
  - Format compatible with large scale sample testing

METHODS



Virus in sample (PFU/mL)	Columns that will contain 15-150 PFU/well*
1E+02	1
1E+03	1-3
1E+04	3-5
1E+05	5-7
1E+06	7-9
1E+07	9-11

** If inoculum volume = 0.15 mL / well and dilution factor = 0.5 Log_{10}*



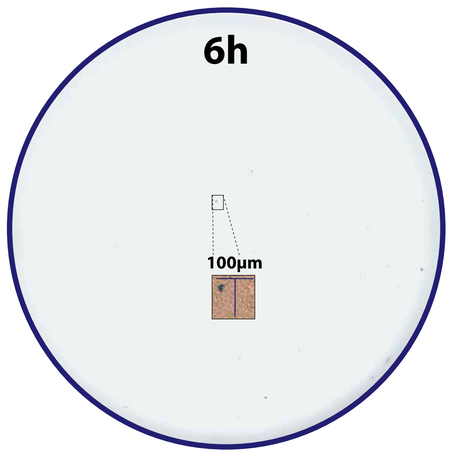
1 DILUTE Inhibitor → 2 DILUTE virus suspension → EXPECT viral load per well → IMMUNOSTAINING

3 COMBINE inhibitor and virus dilutions → 4 INOCULATE permissive cells → 5 INCUBATE at 35 – 37°C → 6 FIX & IMMUNOSTAIN cell monolayer; HARVEST & STORE supernatant at -80°C

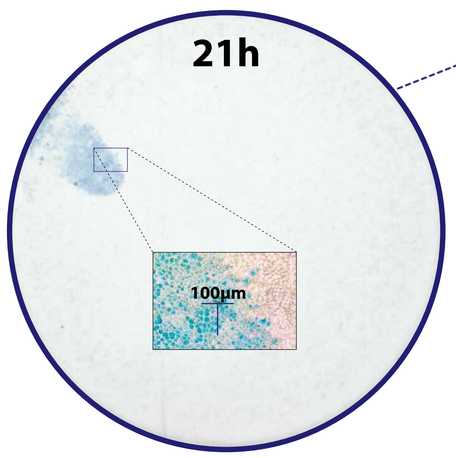
7 CAPTURE images of each well → VISUAL analysis / JPEG or TIFF → IDENTIFY wells containing resistant virus → SELECT supernatants for further analysis

QUANTITATE signal / Biospot software (C.T.L.) → CALCULATE inhibition (% of maximal virus signal in absence of inhibitor)

RESULTS

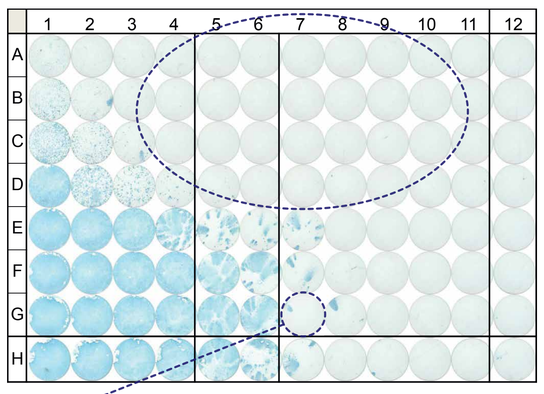


- ONE spot showing ONE infected cell
- TOO EARLY to detect secondary infections

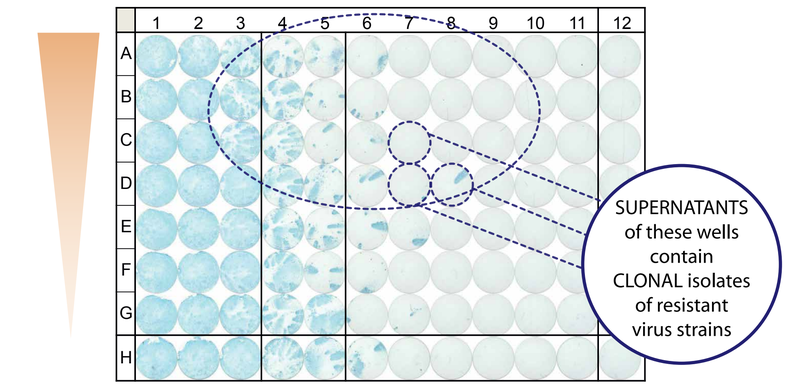


- Monolayer with ONE spot showing that progeny virus of ONE infectious unit infected >>1000 cells within 24 h

Sensitive A(H3N2) virus 21h



Resistant A(H3N2) virus 21h



% INHIBITION of maximal virus signal (row H)

	1	2	3	4	5	6	7	8	9	10	11	12
A	93	98	99	99	100	100	-	-	-	-	-	-
B	81	90	98	100	100	100	-	-	-	-	-	-
C	51	76	89	100	100	100	-	-	-	-	-	-
D	46	48	63	90	97	100	-	-	-	-	-	-
E	26	54	43	8	30	65	-	-	-	-	-	-
F	11	42	30	29	5	9	-	-	-	-	-	-
G	5	18	25	41	-9	-16	-	-	-	-	-	-
H	*11.5*	*10.4*	*7.7*	*6.7*	*5.4*	*4.7*	*1.8*	*0.00*	*0.10*	*0.00*	*0.00*	*0.00*

PFU / Well: > 150 | 15-150 | 0-5 | 0

Too high | Good | Too low

MAXIMAL virus signal for phenotyping

	1	2	3	4	5	6	7	8	9	10	11	12
A	7	-1	-56	18	42	-	-	-	-	-	-	-
B	7	27	-62	-11	71	-	-	-	-	-	-	-
C	31	34	-45	14	78	-	-	-	-	-	-	-
D	14	35	-20	9	33	-	-	-	-	-	-	-
E	-1	34	-25	-6	19	-	-	-	-	-	-	-
F	8	27	-12	0	45	-	-	-	-	-	-	-
G	-11	30	-48	-11	-19	-	-	-	-	-	-	-
H	*5.0*	*5.0*	*3.2*	*4.5*	*3.9*	*2.2*	*0.1*	*0.01*	*0.00*	*0.01*	*0.00*	*0.00*

PFU / Well: > 150 | 15-150 | 0-5 | 0

Too high | Good | Too low

INHIBITION
- 90 - 100 %
- 50 - 90 %
- 0 - 50 %
- \- < 5 PFU/well in virus control

CONCLUSIONS

- **This phenotypic ViroSpot™ Assay allows RAPID DETECTION and characterization of virus in samples containing 1E2 – 1E7 PFU/mL.**
- **Progeny virus of RESISTANT virus clones, which were cultured in the PRESENCE of inhibitor, can be ISOLATED and stored for further analyses.**
- **Since prior culture in absence of inhibitors is not required, this assay may provide valuable PHENOTYPIC information on HETEROGENEOUS VIRUS POPULATIONS in clinical specimens.**