Influenza virus and RSV ViroSpot™ Assays for high-throughput virology testing

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INTRODUCTION

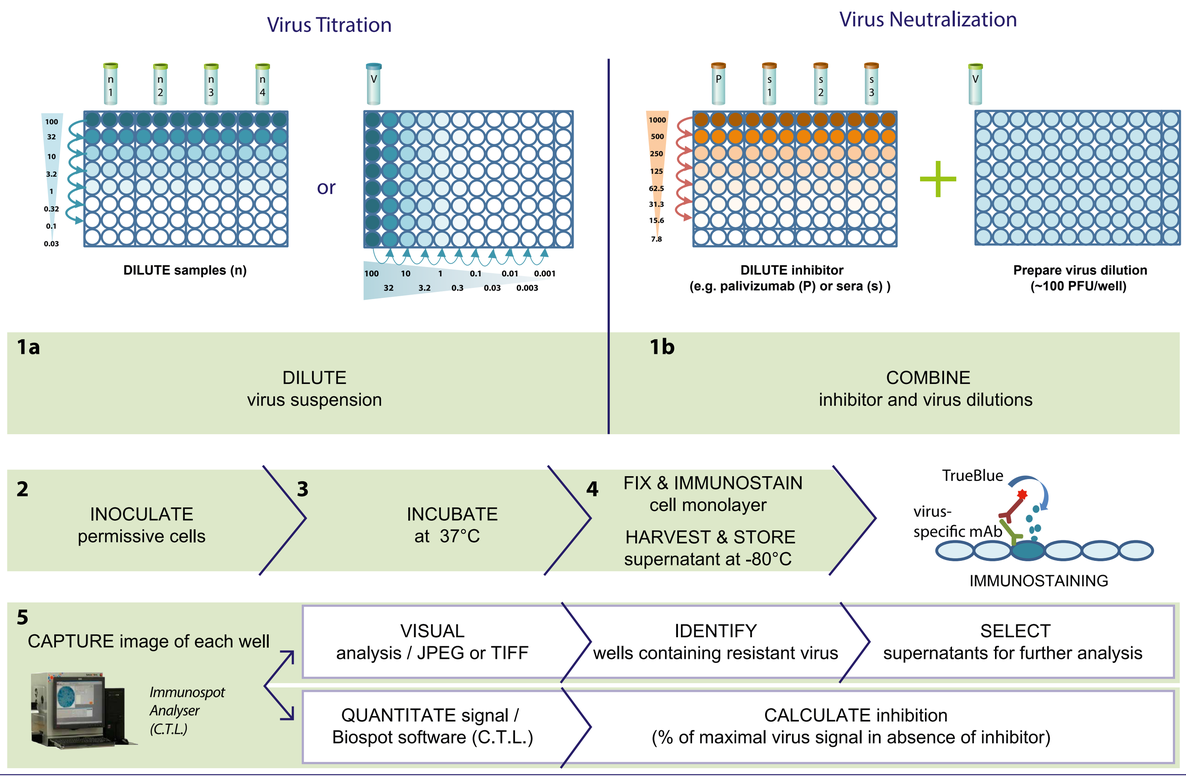
Large-scale sample analyses in phase II and III clinical trials of novel vaccines and antiviral compounds require objective and reliable methods for high-throughput virus detection, quantitation and characterization.

Compared to molecular readouts, this is generally more difficult for virus culture assays.

Here we developed influenza virus and RSV ViroSpot assays, which combine CLASSIC VIRUS CULTURE techniques with AUTOMATED SENSITIVE detection of immunostained virus-infected cells in 96-well microtiter plates FOR:

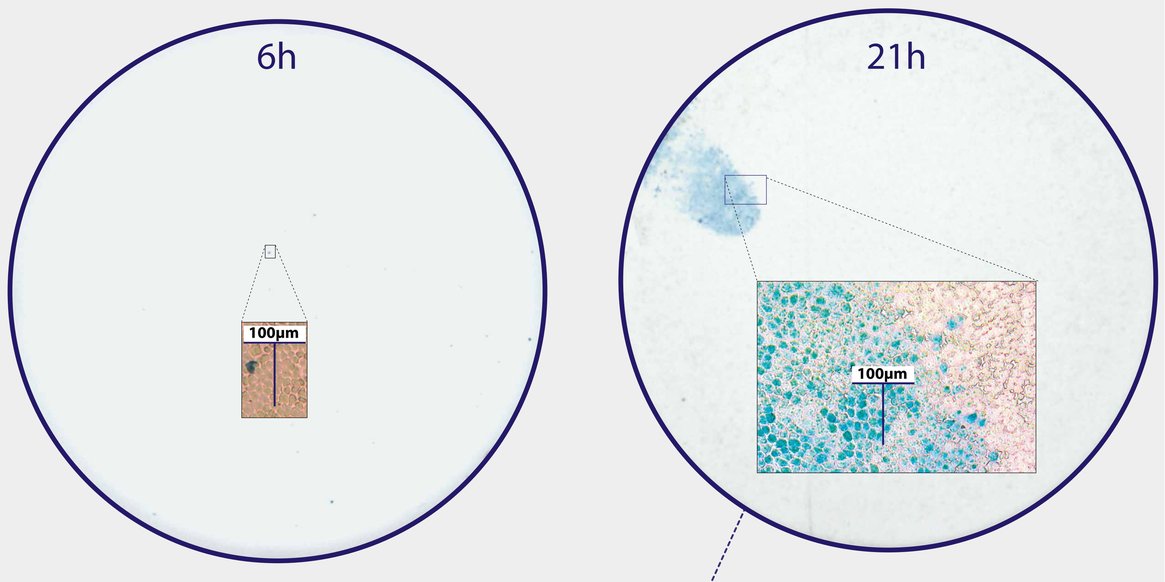
- virus TITRATION: PFU/mL or $TCID_{50}$/mL
- virus NEUTRALIZATION: antibody neutralization titer
- virus PHENOTYPING: IC_{50} and IC_{90}
- drug-RESISTANCE MONITORING: isolation escape variants from primary specimens

METHODS



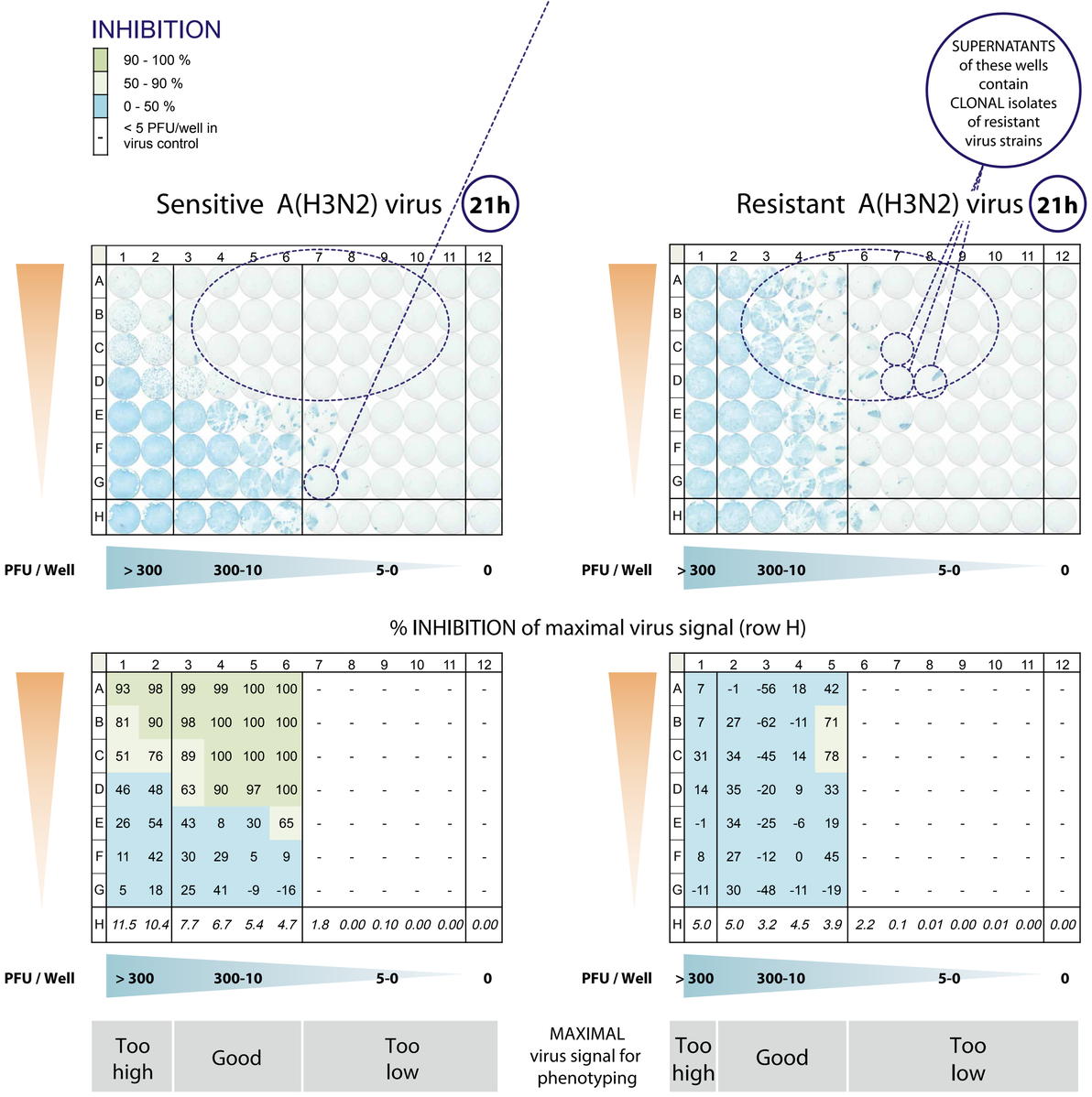
RESULTS

Influenza virus



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

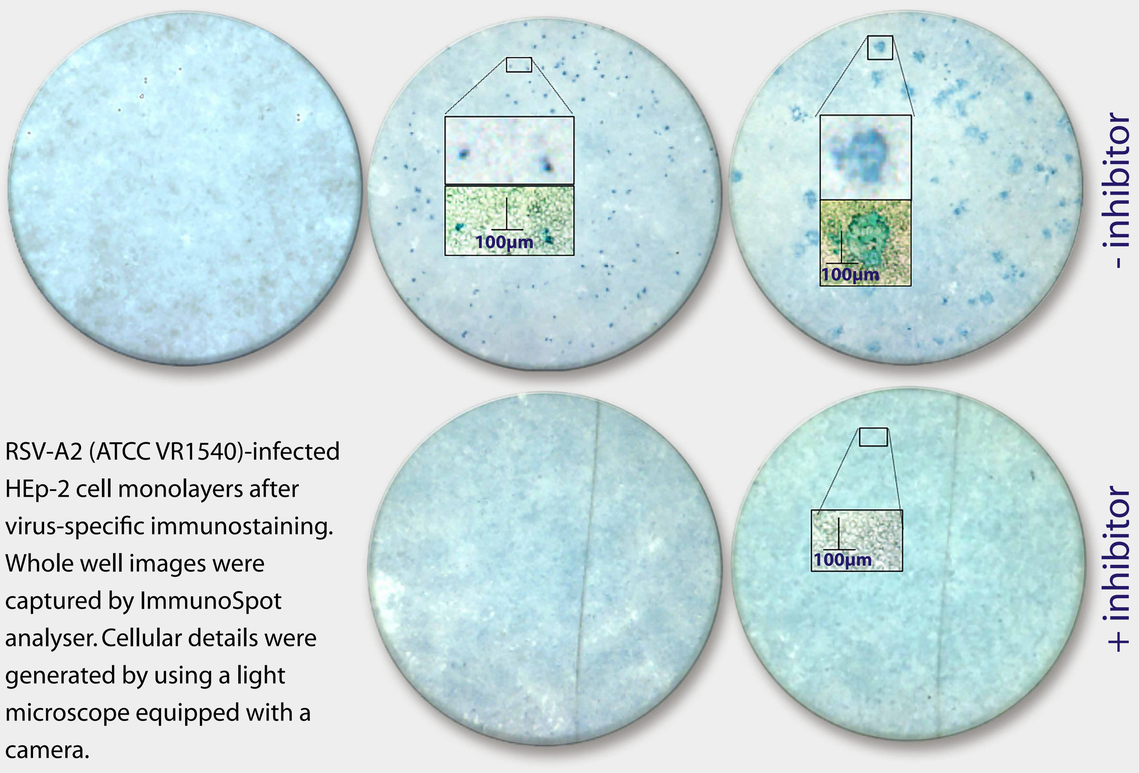
- Monolayer with ONE spot showing that progeny virus of ONE infectious unit infected >>1000 cells within 24 h



	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*

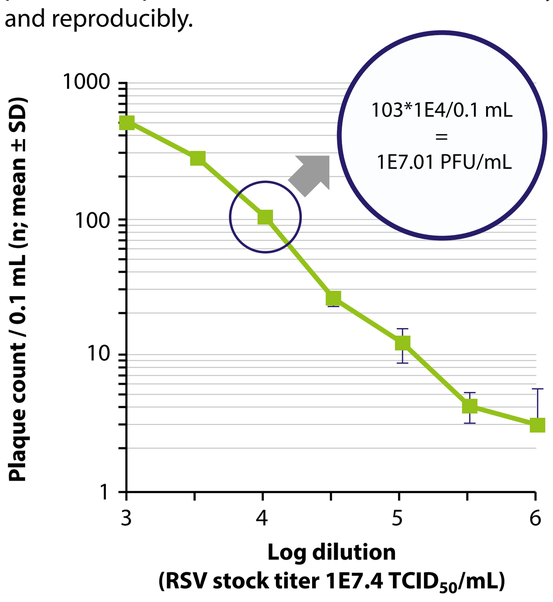
	1	2	3	4	5	6	7	8	9	10	11	12
A	7	-1	-56	18	42	-	-	-	-	-	-	-
B	7	27	-62	-11	71	-	-	-	-	-	-	-
C	31	34	-45	14	78	-	-	-	-	-	-	-
D	14	35	-20	9	33	-	-	-	-	-	-	-
E	-1	34	-25	-6	19	-	-	-	-	-	-	-
F	8	27	-12	0	45	-	-	-	-	-	-	-
G	-11	30	-48	-11	-19	-	-	-	-	-	-	-
H	*5.0*	*5.0*	*3.2*	*4.5*	*3.9*	*2.2*	*0.1*	*0.01*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

RSV

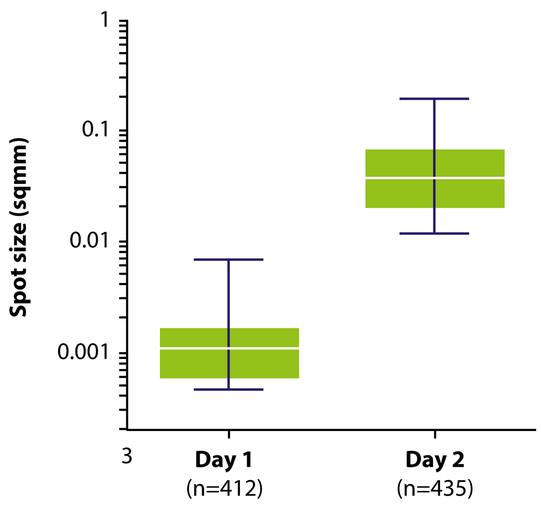


RSV-A2 (ATCC VR1540)-infected HEp-2 cell monolayers after virus-specific immunostaining. Whole well images were captured by ImmunoSpot analyser. Cellular details were generated by using a light microscope equipped with a camera.

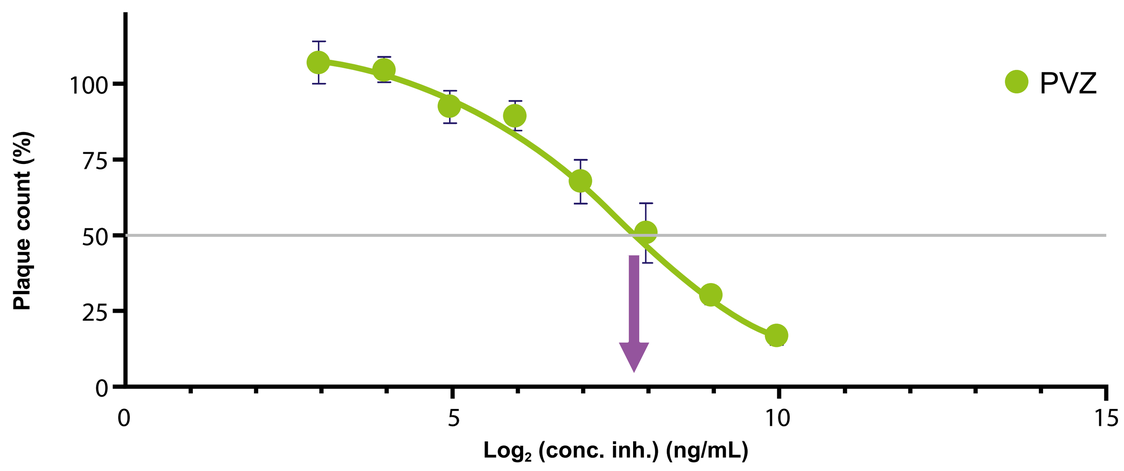
At day 1, RSV-infected cells ranging from 30 to 300 per well (4 replicates), could be COUNTED efficiently and reproducibly.



Medium PLAQUE SIZE increased ~ 40-fold between day 1 and day 2: from 0.001 mm^2 to 0.040 mm^2.



Palivizumab-mediated RSV NEUTRALIZATION, day 1 readout: IC_{50} for RSV A2 was 2E7.7 ng/mL = 208 ng/mL.



DISCUSSION/CONCLUSION

- The **SENSITIVITY** of detection at the **SINGLE CELL** level omitted the need for prior virus amplification in absence of inhibitors. This facilitates **DIRECT PHENOTYPIC** analyses of heterogeneous virus populations in **CLINICAL SPECIMENS.**
- Because **SOLIDIFYING** reagents, such as agarose, avicel or methyl cellulose, were **NOT NECESSARY**, cognate **VIRUS YIELDS** could be stored for **GENOTYPING** and further **PHENOTYPIC** analyses.
- Propagation of **RESISTANT MINORITY** species in presence of selective inhibitors was **READILY DETECTED.**
- Counting **INDIVIDUAL INFECTED CELLS**, plaques or infected areas in 96-well plates provided values that were directly **PROPORTIONAL TO VIRUS PROPAGATION.**
- **INCUBATION PERIODS** for virus titration, virus neutralization and inhibition assays were **SIGNIFICANTLY REDUCED** compared to conventional techniques, while traceability of raw data and **OBJECTIVITY** of results **IMPROVED.**