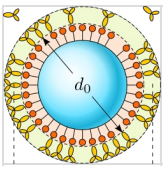

Sep 06, 2020

HolographicDiagnostics: Automated Virus Binding Assay

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1 Works for me dx.doi.org/10.17504/protocols.io.bkpgkvjw

Coronavirus Method Development Community Grier Group 2 more workspaces

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ABSTRACT

This protocol describes the steps required to perform a holographic immunoassay for SARS-CoV-2 whole virus particles. The assay uses an xSight holographic particle characterization instrument (Spheryx, Inc.) to monitor the diameter of specifically functionalized probe beads. The diameter of the beads increases by a few nanometers as targets bind to the surface. xSight detects and reports this change, yielding an estimate for the concentration of analytes in the sample. The method for holographic immunoassays (applied to antibody binding assays) is described in

Y. Zagzag, M. F. Soddu, A. D. Hollingsworth and D. G. Grier, Holographic molecular binding assays, *Scientific Reports* **10**, 1932 (2020) and K. Snyder, R. Quddus, A. D. Hollingsworth and K. Kirshenbaum, Holographic Immunoassays: Direct Detection of Antibodies Binding to Colloidal Spheres, submitted for publication (2020).

DOI

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PROTOCOL CITATION

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KEYWORDS

Immunoassay, Holographic Particle Characterization, SARS-CoV-2

IMAGE ATTRIBUTION

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MATERIALS

NAME	CATALOG #	VENDOR

NAME	CATALOG #	VENDOR
HD Test Kit	HD SARS-CoV-2 TK-1	

EQUIPMENT

NAME	CATALOG #	VENDOR
SDNA-1000	NA	
xStream	xStream	Spheryx, Inc.
xCell	xCell-8	
xSight	xSight	

SAFETY WARNINGS

Patient samples must be handled and disposed of appropriately.

DISCLAIMER:

BEFORE STARTING

Sealed patient samples must be heat-treated to inactivate viruses in patient samples before samples are processed in a BSL2 facility.

Sample Collection 10m

10m

1 **2 mL saliva** **$2**

Subject passively collects saliva for testing.

SDNA-1000
Whole Saliva Collection Kit
Spectrum Solutions **NA**

Sample Preparation 1h 45m

30m

2 **65 °C**

Heat treat patient sample to inactivate virus.

Patient samples must remain sealed until they are heat treated. BSL3 facilities are required to work with untreated samples. Heat-treated samples may be opened for processing in BSL2 facilities.

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Heat treatment may disrupt virus particles, liberating the antigens that are targets for the assay. This is not a problem because holographic immunoassays can detect dissolved proteins as well as dispersed virus particles.

3 **20 µl HD SARS-CoV-2 test kit** **$1** 31m

Add HolographicDiagnostics test kit to inactivated saliva sample.

4 **120 rpm, Room temperature** 1h 31m

Incubate sample with test kit.

5 **1000 rpm, Room temperature** 1h 45m

Concentrate test beads by centrifugation.

6 Transfer sample to 96-well plate

Sample barcode must be associated with the well in analytical software.
Currently, this transfer is performed manually using a barcode scanner.
Adoption of an automated sample preparation system also will automate this step

Sample Analysis 21m

7 Transfer 96-well plate to xStream sample robot

xStream
Sample handling robot
Spheryx **xStream**

8 For each sample in 96-well plate, xStream transfers sample to xCell microfluidic chip for measurement in xSight. 1m

xCell
Microfluidic sample holder
Spheryx **xCell-8**

30 µl **$40/8 tests**

Transfer concentrated beads to first available reservoir in xCell.

xCell has 8 reservoirs, permitting up to 8 independent tests to be performed with a single chip. The cost per

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chip will decrease with volume purchases.
xStream automatically loads and disposes of xCells as needed.

9 Perform holographic characterization measurement on loaded sample 18m

xSight
Holographic Particle Characterizer
Spheryx **xSight**

xStream inserts xCell in xSight and initiates sample analysis

xSight settings
wavelength: 445 nm
analytical volume: 1 uL
presaved ROI for each type of test bead in test kit

10 Results of test are reported out. 20m

Report population mean diameter for each type of bead in test kit
HD SARS-CoV-2 TK-1 includes beads for three biomarkers (SARS-CoV-2 S1, S2 and M) and one negative control (H1N1).

Sample Disposal

11 Dispose of used sample containers, pipette tips and xCells

Used sample containers, pipette tips and xCells must be disposed in compliant biohazard containers.
xStream deposits used xCells in suitable containers. Manual disposal is required for all other consumable items.

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