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November 3, 2022

Rochelle P. Walensky, MD, MPH
Director
Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30329

Dr. Walensky:

We write to request information about the flawed CDC diagnostic testing procedures for monkeypox virus. This follows a similar CDC breakdown during the pandemic response when CDC distributed faulty and contaminated COVID-19 test kits in February 2020.

In May 2022, the World Health Organization confirmed a multi-country monkeypox outbreak in non-endemic countries, including the U.S.[1] At that time, it appears that the CDC did not have diagnostic test information specific for monkeypox. However, the CDC published real-time (RT) PCR (Polymerase Chain Reaction) test procedures on May 30, 2022, for a broader non-variola orthopoxvirus test to help detect monkeypox and other orthopoxviruses.[2]

CDC then worked to facilitate diagnostic testing specific for monkeypox. On June 6, 2022, CDC published a RT-PCR test procedure to detect the monkeypox virus.[3] This procedure was intended for international partners and any laboratories interested in pursuing a Laboratory

[1] World Health Organization, *Monkeypox outbreak 2022- global, Overview* (2022) https://www.who.int/emergencies/situations/monkeypox-oubreak-2022
[2] Centers for Disease Control and Prevention Laboratory Outreach Communication System (LOCS), *06/02/2022: Lab Advisory: CDC Publishes Non-variola Orthopoxvirus PCR Testing Procedure,* https://www.cdc.gov/locs/2022/06-02-2022-lab-advisory-CDC_Publishes_Non-variola_Orthopoxvirus_PCR_Testing_Procedure html
[3]Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, (PRB), *Test Procedure: Monkeypox virus Generic Real-Time PCR Test* (June 6, 2022), https://www.cdc.gov/poxvirus/monkeypox/pdf/PCR-Diagnostic-Protocol-508.pdf

Developed Test (LDT).[4] This procedure included sequence information for primer and probe[5] development and cycling conditions. This assay was designed to specifically detect monkeypox virus. The information CDC posted appears to show that the assay only targeted one region of the monkeypox genome. However, diagnostic tests for viruses of concern often target multiple regions to mitigate against possible impediments to one of the targets. Even the troubled CDC COVID-19 test kits originally had three targets.[6]

Less than two months after posting this information, on September 2, 2022, CDC posted the following Laboratory Alert:

> CDC is aware of three *Monkeypox virus* (MPXV) cases in California in which preliminary data show a significant deletion in the tumor necrosis factor (TNF) receptor gene. This gene is the target for the CDC West African MPXV and Generic MPXV real-time PCR tests. At this point, the TNF receptor gene deletion is rare. Molecular laboratory developed tests (LDTs) designed using the CDC published primers and probes that specifically target *Monkeypox virus* did **NOT** detect the virus because of the TNF receptor gene deletion in these specimens. These cases were still correctly diagnosed because they were also tested with an LDT that was developed based on CDC's published non-variola *Orthopoxvirus* (NVO) test. (Bold in original).[7]

A few weeks later, University of Texas Health Science Center at Houston released a pre-print study that analyzed the primer and probe sequences of the CDC recommended monkeypox virus (MPV) generic real-time PCR assay.[8] The study found mismatches in the primer and probe sequences that required rectification to improve detection accuracy.[9]

Given these concerns over flawed monkeypox diagnostic testing, please provide the following by November 17, 2022:

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[4] *Id.*

[5] Probes and primers are two types of single-stranded oligonucleotides used in various types of PCR testing. Probes are used in the detection of specific DNA fragments in qPCR. Primers are used to initiate DNA replication inside the cell and they are also used in the initiation of PCR. *See* E. van Pelt-Verkuil and R. te Witt, *Primers and Probes*, MOLECULAR DIAGNOSTICS 51 (June 4, 2019), https://link.springer.com/chapter/10.1007/978-981-13-1604-3_3

[6] PBS Newshour, *Inside the fall of the CDC* (October 16, 2020), https://www.pbs.org/newshour/health/inside-the-fall-of-the-cdc ("Lindstrom had turned to the lab's expert on coronaviruses to design the U.S. test. They chose one that looked for three targets on the same coronavirus gene.").

[7] Centers for Disease Control and Prevention Laboratory Outreach Communication System (LOCS), *09/02/2022: Lab Alert: MPXV TNF Receptor Gene Deletion May Lead to False Negative Results with Some MPXV Specific LDTs*, https://www.cdc.gov/locs/2022/09-02-2022-lab-alert-MPXV_TNF_Receptor_Gene_Deletion_May_Lead_False_Negative_Results_Some_MPXV_Specific_LDTs.html

[8] Fuqing Wu, *et al*, *Wide mismatches in the sequences of primers and probes for Monkeypox virus diagnostic assays,* MEDRXIV (posted October 6, 2022), https://www medrxiv.org/content/10.1101/2022.08.10.22278644v2

[9] Neha Mathur, *Mismatches in the primer and probe sequences of current Monkeypox virus diagnostic assays require rectification to improve detection accuracy*, NEWS MEDICAL LIFE SCIENCES (October 11, 2022). https://www.news-medical.net/news/20221011/Mismatches-in-the-primer-and-probe-sequences-of-current-Monkeypox-virus-diagnostic-assays-require-rectification-to-improve-detection-accuracy.aspx

1. Why were CDC's published primers and probes specifically targeting Monkeypox unable to detect the virus in some cases?
2. Were the CDC's published primers and probes based on currently circulating monkeypox strains?
3. How did the CDC become aware that its published primers and probes led to false negatives?
4. What action has CDC taken to correct this problem? Is CDC re-designing and reworking its published primers and probes?
5. Is CDC actively performing *in silico* analysis of this assay against the most current monkeypox sequence database?
6. If yes, did CDC observe any potential limitation with the assay to give a potential false negative result?
7. If yes, is CDC developing new signatures and assays to counter the limitations?
8. If yes, when will these new signatures and assays be available to the public health/laboratory community?
9. Has CDC thought about developing RT-PCR assays for multiple regions or loci to enhance sensitivity and eliminate false negative results? If not, why not?
10. Monkeypox virus has two clades. Clade 1 or Congo Basin clade monkeypox virus has about a 10 percent fatality rate in unvaccinated persons. Clade 2 or West African clade monkeypox virus (the version currently circulating in humans) is associated with less than one percent mortality.[10] Does CDC have a monkeypox specific assay that detects both clade 1 and clade 2 viruses? If not, why not?
11. The non-variola orthopoxvirus assay cross-reacts with other viruses. Is this a concern to CDC? If not, why not?

In its response, we request that the CDC reproduce each question with a written response to the particular question. If you have any questions, please contact Alan Slobodin of the Minority Committee staff at 202-225-3641. Thank you for your attention to this request.

Sincerely,

Cathy McMorris Rodgers
Republican Leader
Committee on Energy and Commerce

Brett Guthrie
Republican Leader
Subcommittee on Health

[10]Christina L. Hutson, *et al*, *Dosage Comparison of Congo Basin and West African Strains of Monkeypox Virus using a Prairie Dog Animal Model of Systemic Orthopoxvirus Disease*, 402 VIROLOGY 72-82 (2010). https://www.sciencedirect.com/science/article/pii/S0042682210001650?via%3Dihub

H. Morgan Griffith
Republican Leader
Subcommittee on Oversight and
Investigations

CC: The Honorable Frank Pallone, Chairman
The Honorable Anna Eshoo, Chair, Subcommittee on Health
The Honorable Diana DeGette, Chair, Subcommittee on Oversight and Investigations