

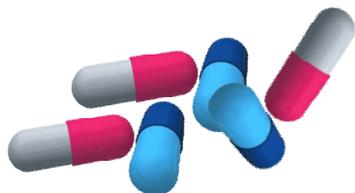


*Thân thiện như chính ngôi nhà của bạn*



# Laboratory Diagnostic Strategies MFHD

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# Overview



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- The virology diagnosis behind a case of HFMD takes on a great significance mainly because of the need to rapidly and accurately determine if the virus is one associated with severe complications, in the case of EV71.
- The clinical hallmarks of HFMD do not distinguish between EV71 and other viruses and confirmation of diagnosis is by laboratory tests





# Overview (tt)



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- In addition, there is a subset of infected patients who do not present with the typical characteristics and present a diagnostic challenge, particularly in the face of the risk of severe.
- Confirmatory diagnosis is based on tissue culture and virus isolation and detection which are both laborious and time-consuming.



# Overview (tt)



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- These methods therefore are not practical for clinical decision-making, particularly in deciding between interventions and treatment in patients where the clinical hallmarks are ambiguous or even absent
- Antibody based tests and PCR are more widely available now and allow for more rapid diagnosis but are not yet widely used due to cost as well as concerns sensitivity and specificity.



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# Overview (tt)



- what would be most useful is a sensitive and specific method for the direct detection of EV71 antigen or RNA.
- Current molecular techniques allow for the genomic analysis of the causal agents and allows for tracking of viruses such as EV71 as well as relationships of the virus strains in the region.



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# Overview (tt)



- For some countries, particularly when the burden of testing is high at the peak of an epidemic, severe cases will be prioritized for rapid diagnosis through Real time RT-PCR for an initial, presumptive diagnosis. Positive cases will be prioritized for virus isolation .

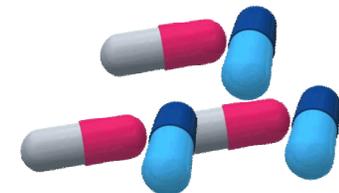


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# Laboratory Detection Methods



- There is no widely available, sensitive rapid diagnostic test for EV 71.
- Laboratory diagnosis of EV71 infection depends on traditional virus isolation , followed by identification of virus serotype by neutralization with monoclonal antibodies, indirect immunofluorescence assay (IFA), or Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).





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# Laboratory Detection Methods

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- Primary specimens that can be sampled for culture include throat swab, vesicle fluid, stool samples, cerebrospinal fluid (CSF) and sera. The yield for both CSF and serum is very low.



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# Rapid presumptive diagnosis on primary specimens

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- The types of testing and diagnostic strategies favored are dependent on resources and capacity.
- Most labs currently depend on direct RT- PCR as the method of choice allowing for the earliest, presumptive diagnosis of EV71.



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## Rapid presumptive diagnosis on primary specimens

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Sensitivity of detection between methods used on EV71 **isolates** and direct specimen RT- PCR are both high and comparable and so currently this is considered is the most rapid method for detecting EV and EV-71 in suspect samples before selecting positive samples for virus isolation and confirmatory sequencing.



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## Rapid presumptive diagnosis on primary specimens

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EV71 IgM-specific enzyme-linked immunosorbent assay (ELISA):

Assays are being developed but still most have problem with specificity for EV71 resulting in many false positive results and giving the tests low positive predictive value for EV71 infection.



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# Type of Specimen



- One of the challenges of diagnosis is the type of specimen available for virus detection.
- Throat and vesicle (if available) swab samples in virus transportation medium (VTM) are considered to be the most useful specimens in terms of the rate of virus detection of HFMD



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# Type of Specimen



- EV71 can be shed in the stool for several weeks and stool (rectal swab) samples are also appropriate clinical specimens for virus detection and/or isolation.
- To increase the possibility of enterovirus detection, collecting throat swab samples for all patients plus swabs from at least two vesicles or from the rectum for patients with no vesicles is recommended.



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# Type of Specimen

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1. Stool: Ad: Easy to collect. Long duration of virus excretion, High rate of positivity  
(Dis: For in patients only, Collection time depends on patient)
2. Throat swab in Virus Transport Medium (VTM): Ad: Easy to collect, For out patients  
(Dis: Need well train collector, Short duration of viral shedding )



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## Type of Specimen



3. Vesicular fluid in VTM: Ad: Virus obtained from the fluid is a causative agent, High rate of positivity (Dis: Difficult to collect vesicular fluid from very small vesicles ).
4. Rectal swab : Ad: Available for all patients (Dis: Not sterile site, Isolation of virus there might represent coincidental asymptomatic carriage rather than the causative agent )



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# Type of Specimen

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## 5. Cerebrospinal fluid:

The virus detection rates of EV71 and CA16 from cerebrospinal fluid (CSF) samples are rather low (less than 5%), Good to determine specific causative agent of viral meningitis. (Need experienced doctors to do lumbar puncture, Highly insensitive for isolating EV71)



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## Sources of documents

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- *Basic Laboratory Procedure in Clinical Microbiology (WHO) - Ebook*
- *HFMD Forum Final Report for publishing 4.12.2009 (CDC) – Ebook*
- <http://www.apnet.org.au/>



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# Thông Báo triển khai XN

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- Nhằm đáp ứng nhu cầu chẩn đoán và điều trị bệnh tay chân miệng tại BV.
- Khoa Vi sinh dự kiến triển khai xét nghiệm **Realtime RT-PCR *Enterovirus* subtype 71.**
  - + Thời gian bắt đầu thực hiện: 23/06/2011.
  - + Giá thành:  $\geq$  600.000 đồng/mẫu



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# Thông báo

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+ Thời gian trả kết quả xét nghiệm:  
trong vòng 24 giờ sau khi nhận mẫu.

+ Mẫu bệnh phẩm yêu cầu: **phết họng, mũi, bóng nước, trực tràng** và dịch não tủy (khi có viêm màng não vi rus).

+ Mẫu được bảo quản ở 2 -8 C ngay và vận chuyển ngay đến khoa Vi sinh bằng thùng đá lạnh.



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“Knowing is not enough, we must apply  
Willing is not enough, we must do”

