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Detecting cytomegalovirus shedding in pregnant women and newborns: are all swabs created equal?

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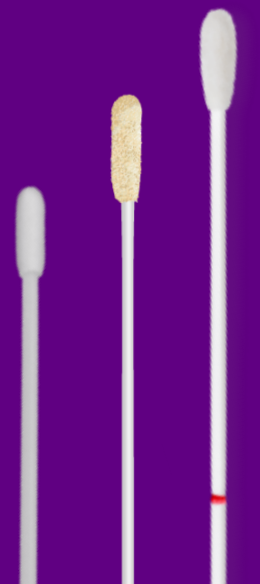
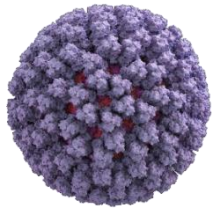
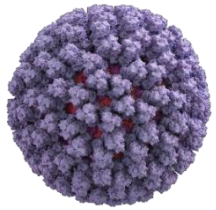


CMV infection in pregnancy



- Ubiquitous virus. Found in saliva, blood, urine, body fluids.
- In healthy people, CMV infections do not usually have symptoms. Some have mild symptoms.
- *But*, CMV can cause significant and long-term health issues – cCMV and SNHL.
- 3416 infants born with cCMV (UK, 2020); 629 suffer from permanent disability.
- €853,000,000 pa.
- Detecting maternal and fetal infections is essential.

CMV infection in pregnancy



- Current approach
  - Screening?
  - USS ± MRI
  - Serology ± PCR
    - M/F blood
    - Amniotic fluid
  - PCR
    - Urine ± saliva
    - DBS
- Proactive approach?
  - Numerous studies
  - Detecting CMV shedding in maternal body fluids by PCR
    - Urine
    - Vaginal fluid
    - Saliva

Cytomegalovirus Shedding Characteristics in Pregnant Women (cCHIPS)

ClinicalTrials.gov Identifier: NCT04021628

Recruitment Status : Active, not recruiting
First Posted : July 16, 2019
Last Update Posted : July 21, 2021

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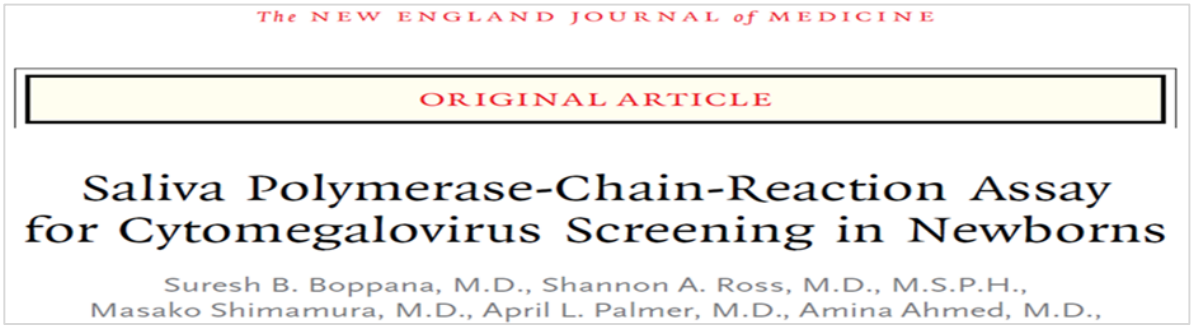

- Saliva from 34,989 newborns.
- Saliva culture vs wet swabs (phase I) and dry swabs (phase II).
- Concurrent saliva culture and PCR of wet and dry swabs for 5276 samples.
- Polyester (PurFybr) swabs in culture media stored at 4°C.
- Polyester dry swabs stored at RT. Direct PCR of dry swabs (not extracted).

Table 2. Real-Time Polymerase-Chain-Reaction (PCR) Assays of Liquid- and Dried-Saliva Specimens, vs. Rapid Culture, Used to Screen for Congenital Cytomegalovirus Infection.

Rapid Culture	Liquid-Saliva PCR Assa		
	Positive	Negative	T
Positive	85	0	
Negative	8	17,569	1
Total	93	17,569	1
Sensitivity (95% CI) — %	100 (95.8–100)		
Specificity (95% CI) — %	99.9 (99.9–100)		
Positive likelihood ratio (95% CI)	2197 (1099–4393)		
Negative likelihood ratio (95% CI)	0 (0.0–0.1)		
Positive predictive value (95% CI) — %	91.4 (83.8–96.2)		
Negative predictive value (95% CI) — %	100 (99.9–100)		

COMPARISON OF SALIVA PCR ASSAY VERSUS RAPID CULTURE FOR DETECTION OF CONGENITAL CYTOMEGALOVIRUS INFECTION

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April L. Palmer, MD,† Amina Ahmed, MD,‡
Robert W. Tolan, Jr., MD,§ David I. Bernstein, MD,¶
*Marian G. Michaels, MD,‖ Pablo J. Sánchez, MD,***
Karen B. Fowler, DrPH, and Suresh B. Boppana, MD,**
for the National Institute on Deafness and Other Communication Disorders CMV and Hearing Multicenter Screening (CHIMES) Study

- Reliability?
- Applicability?

Viral Load in Discordant Specimens

The median viral load was not significantly different between discordant (1.86×10^5 IU/mL; range: 6.4×10^2 to 4.8×10^7 IU/mL) and concordant (2.5×10^6 IU/mL; range: 1×10^3 to 3×10^{10} IU/mL,

Contents lists available at ScienceDirect

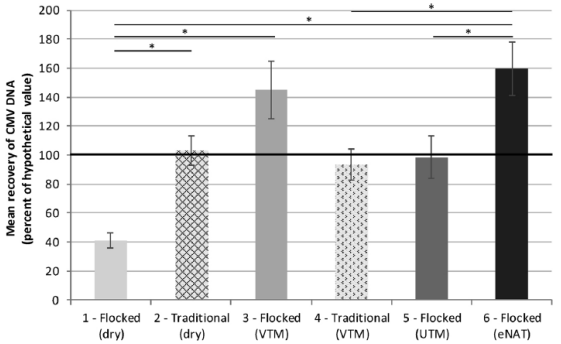
Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Laboratory diagnosis of congenital CMV infection in newborns: Impact of pre-analytic factors

N. Kohmer[a], A. Nagel[b], A. Berger[a], M. Enders[c], K. Hamprecht[d], K. Korn[b], M. Kortenbusch[a], K. Überla[b], H.F. Rabenau[a,*]

- CMV DNA in phosphate-buffered saline.
- Dry flocked and rayon, wet flocked (VTM, UTM, eNAT), wet rayon (VTM).
- Findings
  - The durations and conditions of storage of the swabs (RT; 2h, 24h, 8d) **did not have a major effect** on the recovery of CMV DNA. (but in PBS…)
  - **A significant reduction (60%) in CMV DNA recovery from dry flocked but not rayon swabs.**
    - Not due to under-absorption of flocked swabs.
    - Swab and transport media dependent
- Conclusion: Flocked swabs in VTM or eNAT showed the best recovery, but flocked/eNAT system showed the best overall performance.



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Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Recovery of cytomegalovirus DNA from newborn saliva samples by different methods

Oran Goshen[a,b,*], David M. Goldfarb[a,c], Laura Book[a,c], Peter Tilley[a,c], Soren Gantt[a,b,c]

- CMV DNA in adult saliva.
- Dry polyester and wet flocked (UTM) at RT and 4°C, wet flocked (eNAT) at RT and 42°C.
- Findings
  - **Recovery of CMV DNA is different between <u>different swabs</u>** (0.9 log IU/ml).

Table 2
CMV DNA recovery using various collection and transport systems, time points and storage temperatures.

Swab type	Medium	Storage	Mean log_{10} CMV viral load (standard deviation)				
			Day 0	Day 3	Day 7	Day 14	Day 21
Traditional[a]	None	Room temperature	3.52 (0.10) 4/4	2.54 (0.08)** 2/4	2.21* 1/4	**** 0/4	n.d.
Traditional[a]	None	4 °C	3.52 (0.10) 4/4	3.47 (0.19) 4/4	2.96 (0.13) 4/4	2.42 (0.39) 4/4	n.d.
Flocked[b]	UTM[d]	Room temperature	4.23 (0.04)	2.76 (0.33)	2.56 (0.10)	2.27 (0.17) ***	n.d.
Flocked[b]	UTM[d]	4 °C	4.23 (0.04)	4.00 (0.09)	3.68 (0.18)	2.79 (0.22)	n.d.
Flocked[b]	ENAT[e]	Room temperature	4.40(0.10)	4.53 (0.05)	4.61 (0.07)	4.38 (0.14)	4.47 (0.05)
Flocked[b]	ENAT[e]	42 °C	4.40 (0.10)	4.56 (0.09)	4.60 (0.05)	4.42 (0.11)	4.43 (0.05)
Filter paper[c]	–	Room temperature	3.06 (0.19)	3.30 (0.08)	3.65 (0.09)	2.87 (0.28)	n.d.

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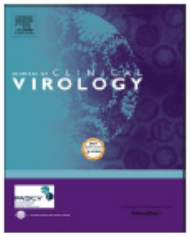


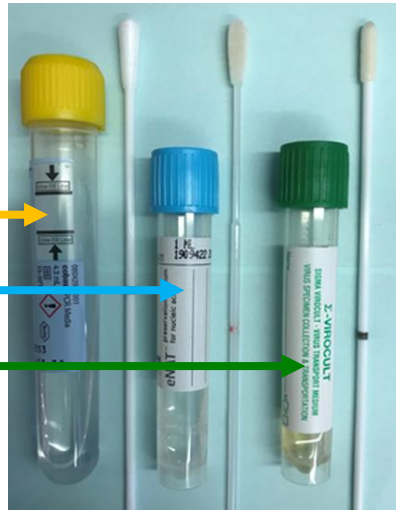
Check for updates

Screening for cytomegalovirus shedding in vagina and saliva: Significant differences between biological fluids, swab types and storage durations in DNA recovery

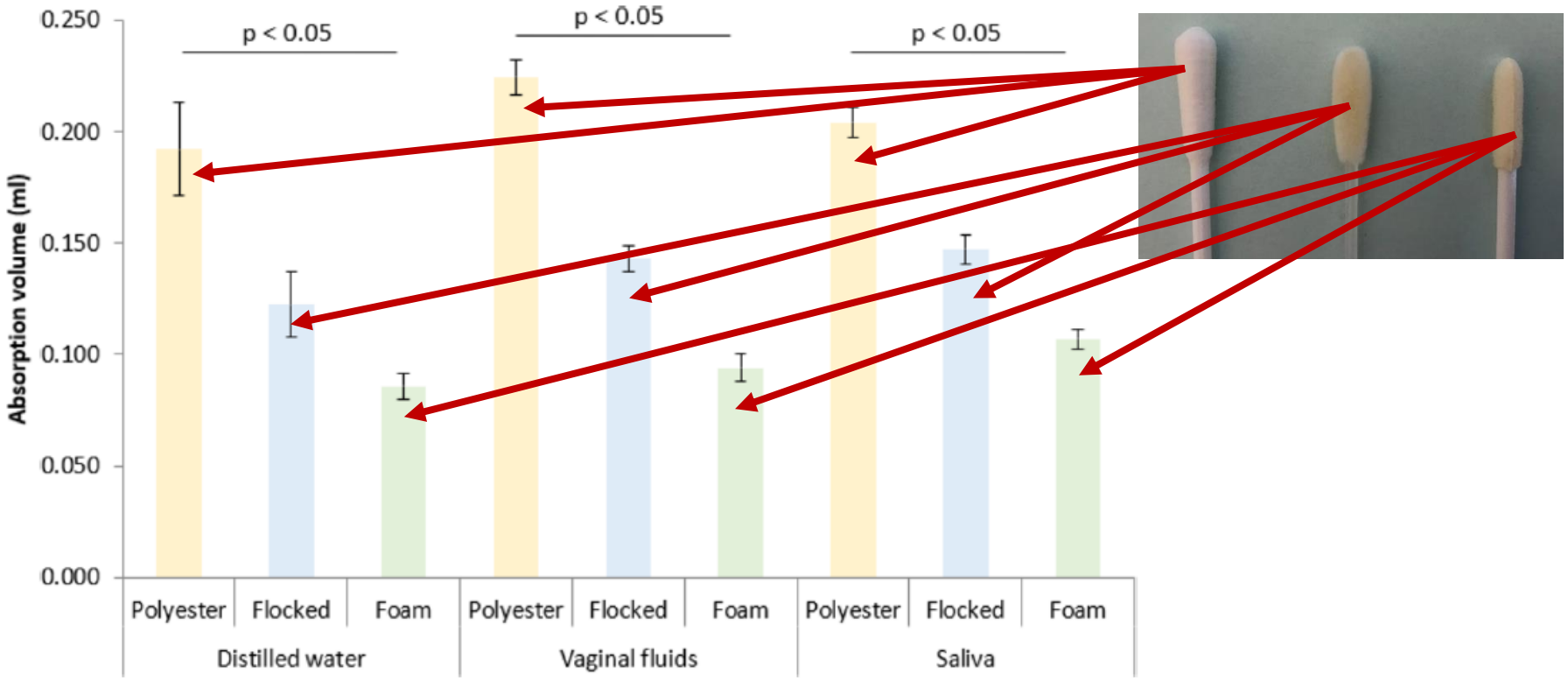
Ngee Keong Tan [a,*], Cassie F. Pope [b,1], David Carrington [b,1]

Evaluation of commercial swabs

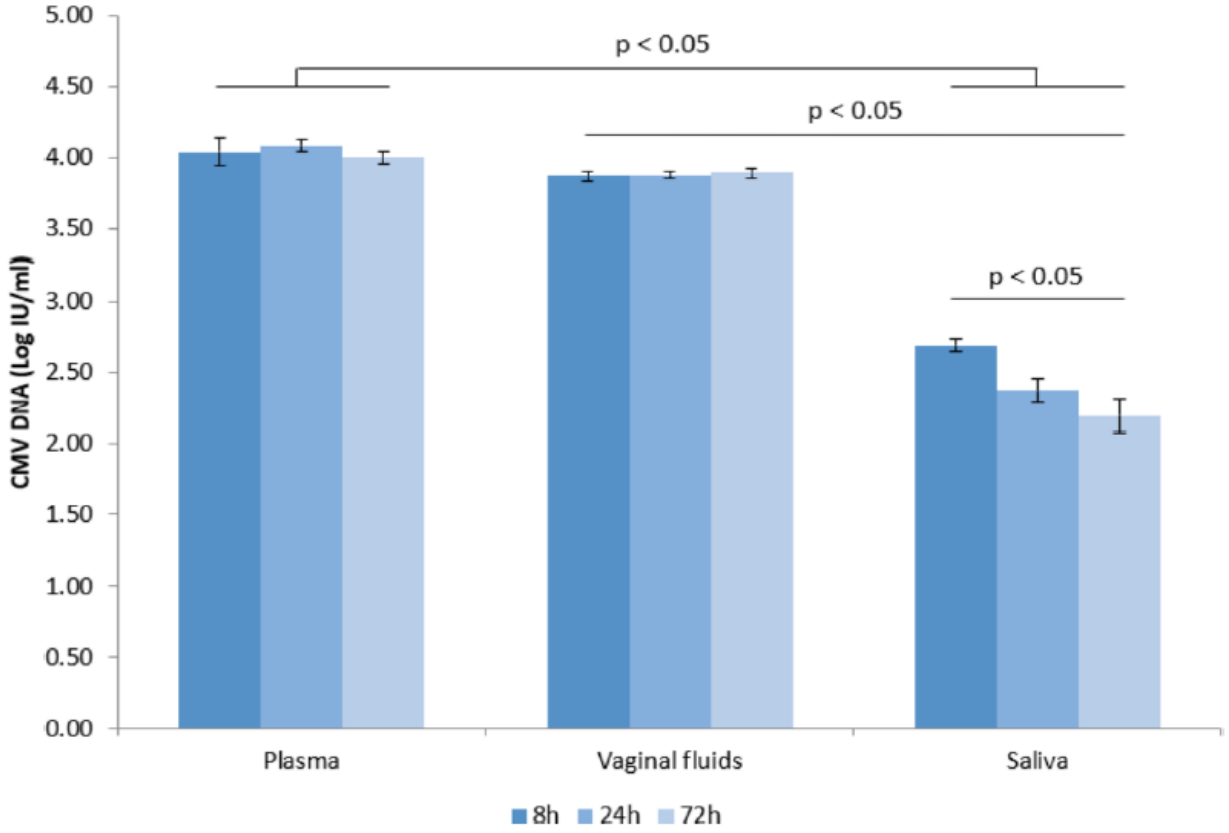
- Adult female vaginal fluid and saliva
  - Polyester, 4.3ml cobas PCR media
  - Flocked (nylon), 1.0ml eNAT media
  - Foam, 1.0ml VTM
  - Foam, dry
- Absorption volume
- Recovery of CMV DNA from fluids over time.
- Recovery of CMV DNA from swabs ± transport media over time.



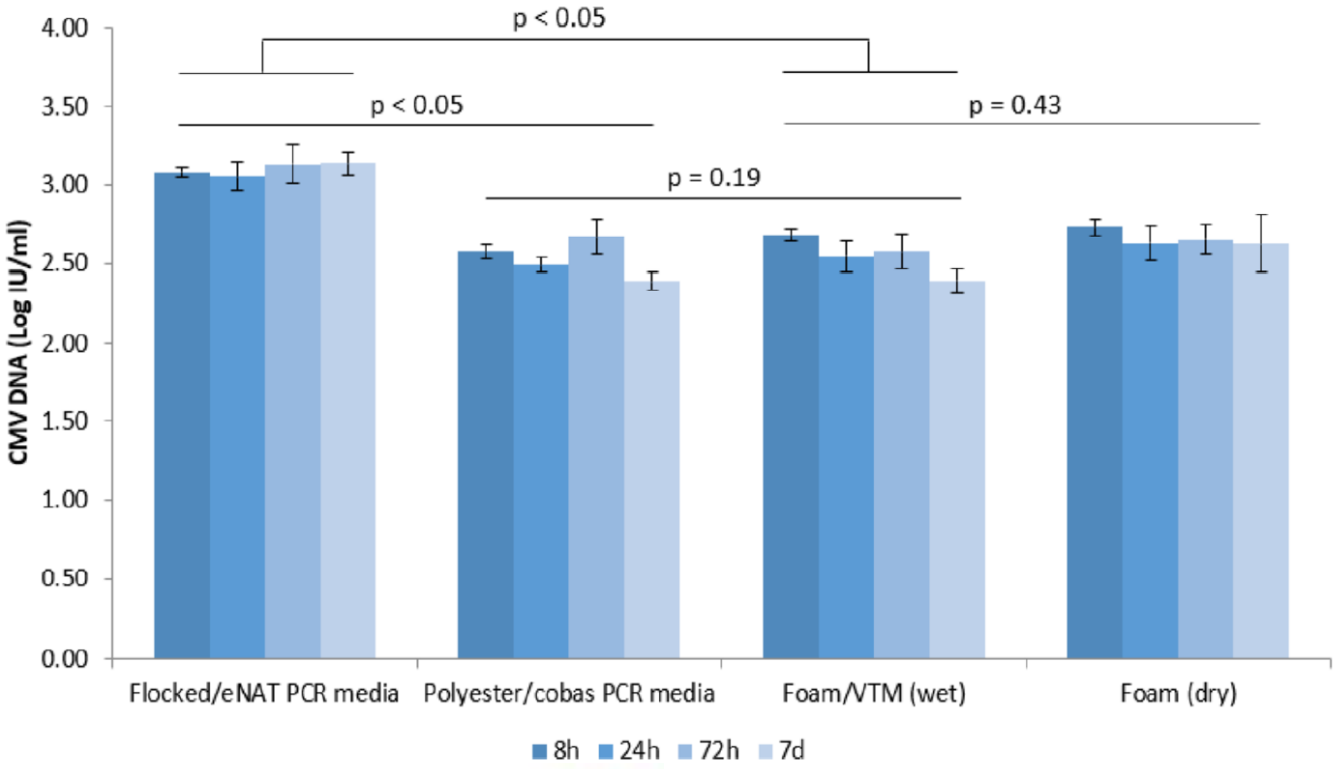
Result 1: Absorption



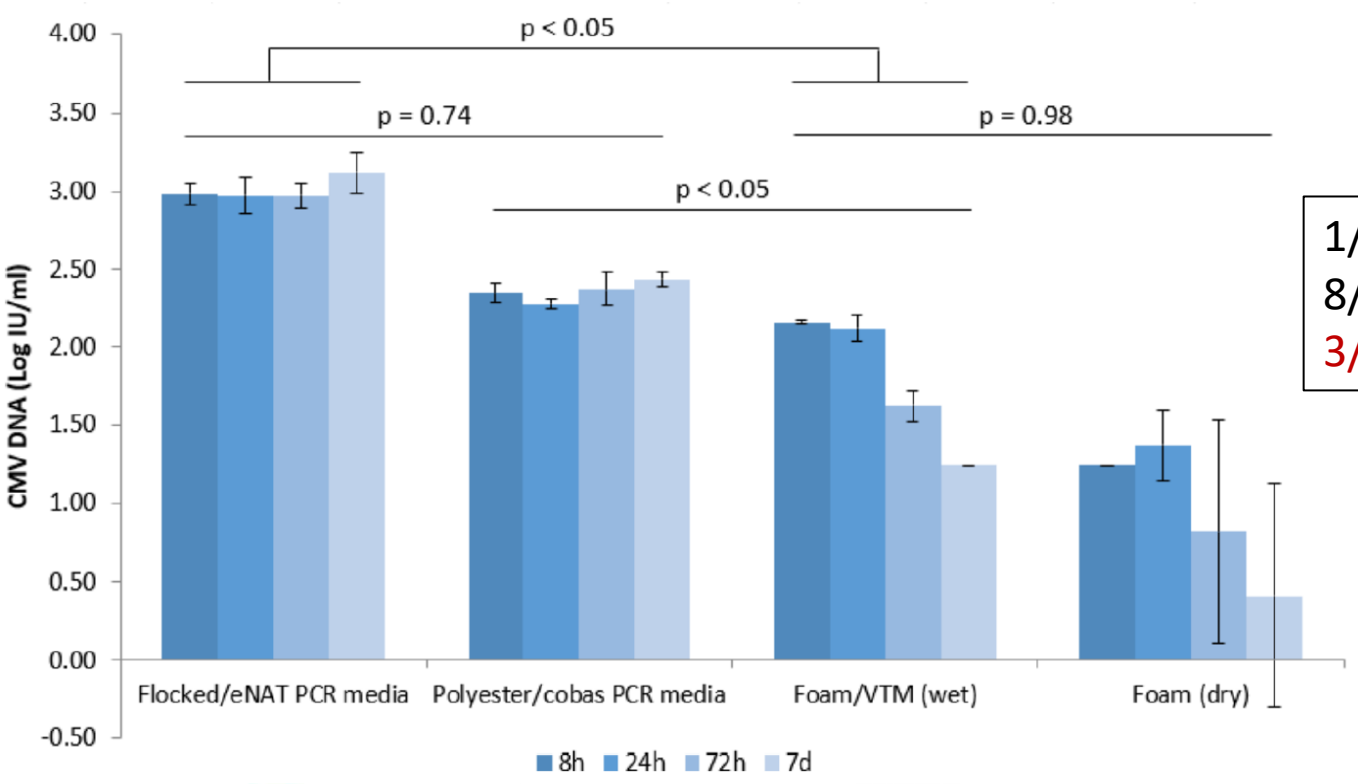
Result 2: Recovery from fluids



Result 3: Recovery from swabs ± TM (VF)



Result 4: Recovery from swabs ± TM (Sal)



1/12 D, Q
8/12 D, NQ
3/12 ND

Conclusion:

- Significant differences in
  - absorption efficiency between swabs.
  - CMV DNA recovery between biological fluids and swab types over time.
- Polyester and flocked swabs with NA preservation media appear acceptable, but flocked swabs in eNAT media are superior for CMV DNA recovery.
- Foam swabs stored dry or in VTM are likely inferior for saliva samples.

Summary and take home message:

- Commercially available swabs, with or without transport media, have **neither equal absorption efficiency, nor the equivalent capability of releasing or preserving CMV DNA over time to ensure an accurate and reliable detection of CMV DNA** in biological fluids.
- Choose your swabs carefully!

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Grazie!