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Revisiting Donor Screening Protocols in Polish Milk Banks: A Pilot Study Supporting the Update of Approaches to Screening Donors with CMV Infection

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TITLE

Revisiting Donor Screening Protocols in Polish Milk Banks: A Pilot Study Supporting the Update of Approaches to Screening Donors with CMV Infection

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ABSTRACT

PURPOSE: Cytomegalovirus (CMV) seroprevalence in Poland is high among women and represents a substantial proportion of milk donors. European Milk Bank Association (EMBA) does not recommend CMV serological screening of donors; Polish milk banks currently require CMV serological testing at recruitment. We evaluated donor screening protocols to align Polish milk bank policies with European SoHO classification by assessing concordance between CMV serostatus-based donor screening and CMV viral DNA detection in breast milk.

METHODS: Twenty lactating mothers were recruited (mean maternal age 30.75 years; mean postpartum time 3.06 months): Donors underwent CMV IgG and IgM serological testing at recruitment. Three milk samples were collected at monthly intervals following a 0-1-2 scheme. Sixty breast milk samples were assessed using qualitative PCR to detect CMV viral DNA. The concordance of CMV presence in serology and an alternate qualitative PCR method was compared using real-time PCR as the reference method.

RESULTS: CMV viral DNA was detected by real-time PCR in 35% of donated milk samples (7/20), with most demonstrating declining viral shedding. The IgM model showed 0% sensitivity, whereas qualitative PCR achieved 82.6% sensitivity. Qualitative PCR had a higher negative predictive value (95.1% vs 68.3%) and overall accuracy (90.0% vs 68.3%).

CONCLUSION: CMV serology-based screening demonstrated limited concordance with CMV viral DNA detection in breast milk, while qualitative PCR-based classification more closely reflected viral DNA presence, it incurred higher operational costs, highlighting biological and operational trade-offs in CMV screening strategies consistent with current EMBA guidance.

Keywords: cytomegalovirus, donor human milk, donor screening, serology testing, milk banks, CMV kinetics

ABBREVIATIONS

Cytomegalovirus – CMV

Confidence intervals – CI's

European Milk Bank Association-EMBA

Substance of Human Origin-SoHO

True positives - TP

False positives -FP

True negatives -TN

False negatives - FN

Positive predictive value - PPV

Negative predictive value - NPV

INTRODUCTION

Human milk is an important source of nutrition for all infants, especially premature infants. When a mother's own milk is unavailable for her newborn, either due to infectious causes, medications or the critical state of the mother, donor human milk plays a crucial role. According to the European Milk Bank Association [EMBA], current donor screening protocol encompasses legal and administrative criteria like age > 18 years, medical interview, informed consent and agreement for processing surplus milk by milk banks. At the same time, extensive clinical screening is also performed with emphasis on transmissible infections like HIV and Hepatitis. EMBA has also stated that there are differences in human milk bank practices within Europe, and in this study, we focus on one such difference - screening protocol for Cytomegalovirus [CMV] (1). CMV establishes life-long latency in its host and can cause serious, debilitating deformities in immunocompromised individuals and premature infants (2). In a recent study, 87.80% of healthcare workers in Poland were IgG seropositive for CMV(3). This also reflects well the seropositivity rates among the general population. The current clinical screening protocol in Poland excludes mothers who are IgM seropositive for CMV or have low IgG avidity (4). Serology does not assess if excluded donors are actively shedding CMV in their breast milk, as the relationship between immune response to CMV and virolactia in breastmilk is poorly characterised in the literature (5). *Yet serology is used in Poland to infer the risk of CMV transmission in breastmilk, highlighting the biological uncertainty used to define the clinical outcome for milk banks in Poland*. It should be considered that different methods are used to prevent CMV transmission through breast milk. Among these, Holder pasteurisation is most widely used but is associated with higher degradation of bioactive elements (6).

AIM

Our study aimed to evaluate the performance of CMV serological screening as a criterion for donor human milk disqualification. We examined whether maternal serological status has concordance with CMV viral shedding in breastmilk, thereby assessing the assumptions underlying current donor screening protocols. The analysis was undertaken in the context of the European Union regulations under the new Substance of Human Origin [SoHO] framework, which states that CMV seropositivity should constitute a contraindication only for non-pasteurised milk (7). Given that serology represents prior exposure rather than active viral presence, we further explored Qualitative PCR detection of CMV viral DNA in breastmilk as a complementary analytical approach to determine whether

direct viral detection more accurately represents transmission risk and better aligns with the SoHO risk-based regulatory model.

MATERIAL AND METHODS

Donor recruitment and informed consent: The study is a retrospective analysis of data from potential donors at the Regional Human Milk Bank in the Holy Family Hospital. As part of the recruitment procedure for milk donation, participants provided informed consent for the use of the donated material as milk and blood for scientific research aimed at optimising milk bank procedures. A total of 20 lactating mothers were recruited according to standard donor recruitment procedures, including serological blood testing. The mean maternal age is 30.75 years, and the mean lactation stage at time of recruitment is 3.06 months postpartum (range 0.46 to 8 months postpartum). The mean gestational age of infants was 37.6 weeks, while the minimum and maximum gestational ages were between 24 and 41 weeks.

Sample acquisition scheme: Peripheral blood samples were obtained from all potential donors at the time of recruitment for CMV serological testing. Baseline serological results were used throughout the donation period, aligning with current guidelines that do not recommend re-testing donors within 3 months (8). CMV IgG and IgM antibodies were measured using the CIMA Architect Abott test in accordance with the manufacturer's instructions. Breast milk samples were collected longitudinally at 3 time points over a period of three months: at recruitment, one month later, and two months later. *These time points were designated as time 1, time 2, and time 3, resulting in a total of 60 samples from 20 donors.* CMV DNA was isolated using the QIAamp DNA Blood Mini kit (QIAGEN). Assay performance was verified to ensure breast milk components did not inhibit the PCR reaction. Qualitative PCR targeting the CMV DNA polymerase gene was performed, and results were recorded as positive or negative. Simultaneously, the milk samples were also verified by quantitative PCR using the Artus ® CMV RG PCR kit from QIAGEN according to the manufacturer's protocol.

Analysis of diagnostic metrics: To evaluate how different screening protocols classify samples in relation to CMV viral detection in breastmilk, both the serology-based donor screening model [Model A]and the qualitative PCR screening model [Model B] were benchmarked using real-time PCR. *Real-time PCR was chosen as the reference method due to its high specificity and ability to quantify CMV viral DNA in breastmilk (9)*. For each screening model, samples were classified as true positives [TP], false positives [FP], true negatives [TN] and false negatives [FN] based on concordance or discordance with the reference method, described in Table 1.

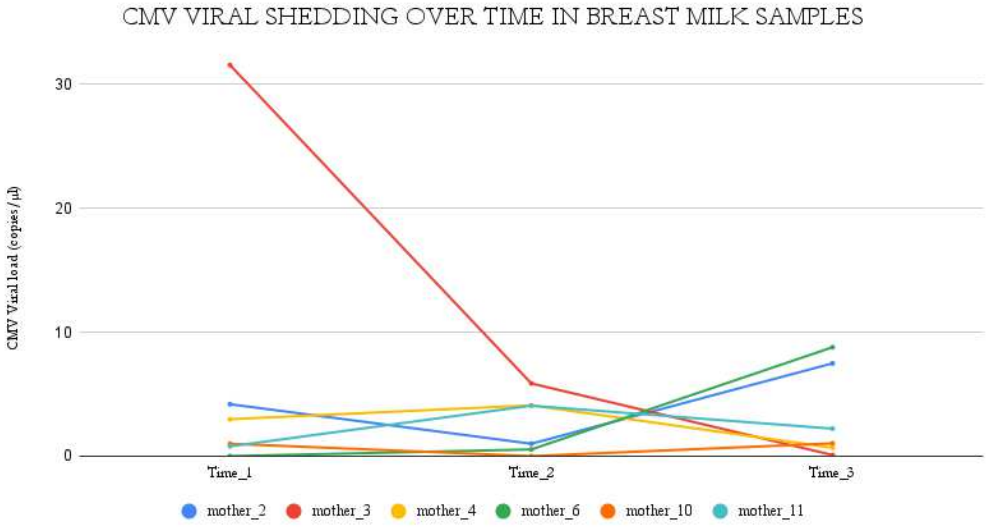
CMV IgG serostatus was defined as positive if ≥ 6.0 IU/ml and negative if <6.0 IU/ml of anti-CMV IgG antibody titres; CMV IgM serostatus was defined as positive if > 0.85 IU/ml and negative if < 0.85 IU/ml of anti-CMV IgM antibody titres; Both in accordance with manufacturer specifications (10). For quantitative real-time PCR, above the manufacturer's limit of detection (~0.3 CMV viral DNA copies/µl) was classified as positive. Failed assays were recorded as invalid. Epidemiologic diagnostic accuracy metrics such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated and defined in Table 2. 95% confidence intervals (CIs) were calculated using the Wilson score method for binomial proportions, better suited for smaller samples. Python and Excel were used for calculations and graph development.

RESULTS

SECTION 1: DONOR SEROSTATUS AND CMV VIRAL SHEDDING PATTERN

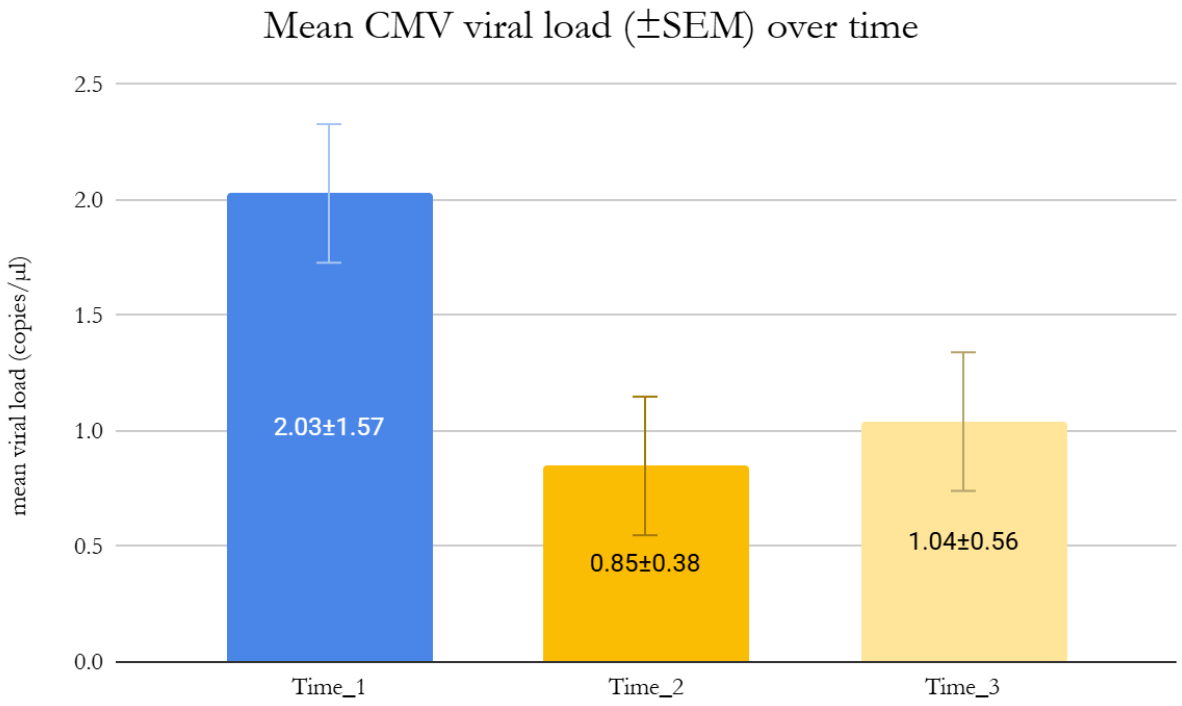
We measured IgG and IgM antibody titres against CMV in peripheral blood samples from donors using serology. 45% of donors were IgG +ve, but none were IgM+ve based on baseline testing. 65% of our population had given birth to full-term infants, constituting the majority, while the remainder gave birth to preterm infants. When considering the breastmilk samples, 35% of donors [n=6] were shedding CMV viral DNA according to real-time PCR in their breastmilk. Figure 1

Fig 1 Interindividual variability of CMV viral DNA shedding in lactating mothers



When comparing overall trends by calculating the average of the viral loads for each time point (Time_1, Time_2 and Time_3), most of these donors actively shedding CMV viral DNA in breastmilk exhibited a decline in viral load over time. The mean CMV viral load at Time_1 was 2.03 copies/µl (SD = 7.04, SEM = 1.57), which sharply fell to 0.85 copies/µl (SD = 1.71, SEM = 0.38) and 1.04 copies/µl (SD = 2.50, SEM = 0.56) at Time_2 and Time_3, respectively, compared to baseline. Figure 2

Fig 2 Mean CMV viral load over time to highlight temporal shedding trends



Though a slight rebound is noticed between time_1 and time_2, statistically significance was limited, given the sample size. *The maximum and minimum viral loads recorded at the time of recruitment were 31.57 copies/µl and 0.79 copies/µl, respectively.*

SECTION 2: CLASSIFICATION PERFORMANCE OF SCREENING MODELS

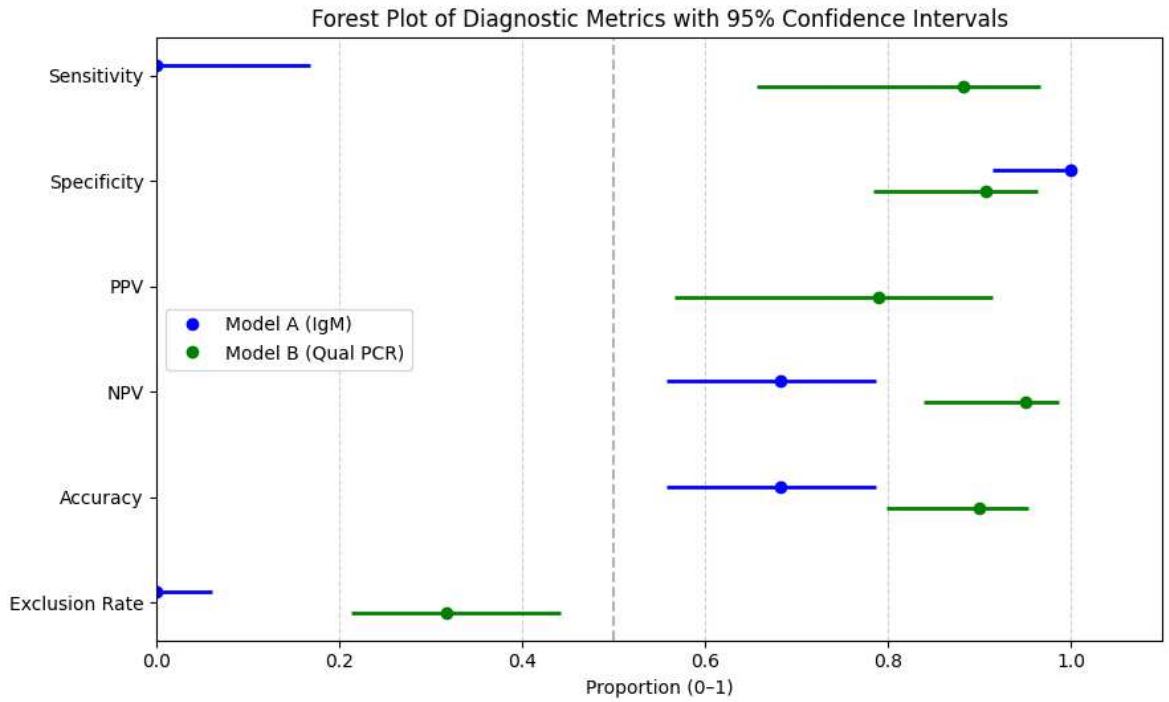
This section reports the classification performance of the predefined IgM-based donor screening protocol [model A] and the qualitative PCR-based screening protocol [model B] relative to CMV viral DNA detection by real-time PCR based on the criteria specified in Tables 1 and 2.

According to real-time PCR, 28.3% of samples across 3 time points had detectable CMV viral DNA copies in breastmilk (>0.3 CMV viral DNA copies/µl). The outcomes and classifications are summarised in Tables 3 and 4. Based on our criteria to assess concordance of CMV viral DNA in breastmilk, the IgM serology model identified 41 samples as true negatives and not shedding CMV viral DNA in breast milk. It classified 19 samples as IgM negative, but these results did not show concurrence with CMV viral DNA recorded by real-time PCR, yielding 19 false negatives. *Model A did not classify any sample as a false positive. The current screening protocol classified no sample as a true positive, i.e., a sample with an IgM positive serology result and concurrence of CMV shedding in breastmilk recorded by the reference method.* This corresponded to a sensitivity of 0% (95% CI, 0.00–14.31%),

specificity of 100% (95% CI, 90.59–100%) for model A. Model A has an undefined positive predictive value (PPV) because it was unable to classify any breast milk samples with CMV viral DNA. Upon considering the ability of the models to identify a negative test result as truly negative based on the negative predictive value, model A has a Negative predictive value (NPV) of 68.3% (95% CI, 55.8–78.7%). Overall, the accuracy of model A was 68.3% (95% CI, 55.8–78.7 %).

The qualitative PCR model identified 15 samples as true positives, i.e., presence of CMV viral DNA in breast milk and confirmed by real-time PCR. The PCR model identified 2 of 60 samples as false negatives in concordance with CMV viral DNA in breast milk as recorded by real-time PCR. Model B had an overall sensitivity of 88.2% (95% CI, 65.7–96.7%) and specificity of 90.7% (95% CI, 78.4–96.3%). Model B has an NPV of 90% (95% CI, 79.9–95.3%). Owing to the number of false positives identified by model B, it has a significantly high exclusion rate of 31.7% (95% CI, 21.3-44.2%). Model B has an accuracy rate of 90.0% (95% CI, 79.9–95.3%). Figure 3

Fig 3 Forest plot representing epidemiological metric as point estimates with 95% Wilson confidence intervals for the overall performance of Model A vs Model B.



DISCUSSION

CMV viral kinetics in breastmilk:

We set out to evaluate the concordance between CMV serostatus and CMV viral DNA detection in human milk to examine how donor classification outcomes vary under different screening approaches. *Our study included 20 donors, the majority of whom were mothers of full-term infants. Most clinical studies of CMV shedding in BM focused on the early postpartum period and mothers of preterm infants, as they are the most at-risk population* (11, 12). *In contrast, CMV shedding kinetics in mature milk, i.e., breast milk produced after approximately 2 weeks postpartum* (13) *from mothers of full-term infants, remains poorly characterised, with authors explicitly noting the lack of longitudinal data in this population* (14, 15). *The incorporation of real-time PCR enabled us to assess the longitudinal viral shedding pattern in the mature lactation stage.* In the majority of cases, CMV shedding in immunocompetent seropositive women exhibits a self-limited course of 8 to 12 weeks postpartum (16) because there is a strong association between mounting IgG-specific immune responses to CMV beyond this timeframe (14). As observed in the literature, we observed an overall decline in CMV shedding. However, we also noted a slight increase in mean viral load between time_2 and time_3, but this was not statistically significant. This nonlinear temporal shedding pattern with time observed in our study is rare, but it does occur, and Azenkot T et al. have recorded CMV viral shedding persisting up to 17 weeks postpartum (14), highlighting the complexity of CMV viral kinetics in HM.

Evaluation of the current donor screening protocol in Poland:

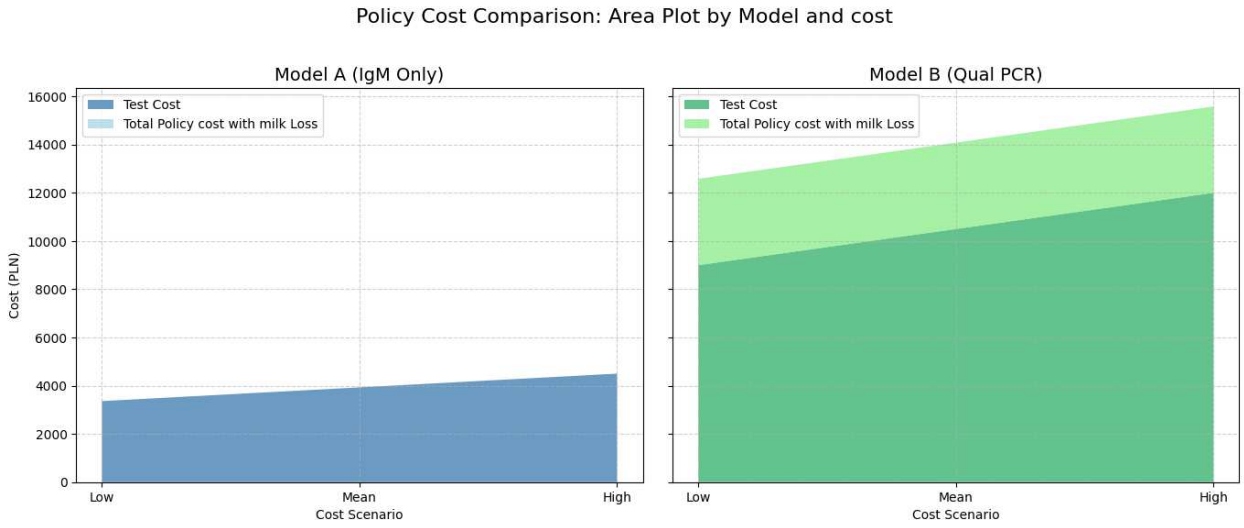
We found that 35% of our population were shedding CMV viral DNA above >0.3 copies of CMV viral DNA copies/µl, with the minimum being 0.7 copies of CMV viral DNA copies/µl. Research highlights that virolactica in BM begins as early as 3 days postpartum in the colostral phase and peaks between 4 and 6 weeks postpartum in the mature milk lactation stage (18,5). Among women with CMV viral DNA detected in breastmilk samples, none had an IgM-positive status by serology, and this is also reflected in the IgM model, i.e., Model A, which has 0% sensitivity. These findings are consistent with the possibility of local CMV reactivation in the mammary glands of donors without systemic seroconversion. Current research echoes the unreliability of depending on negative IgM serostatus to determine the donor pool (11). When qualitative PCR-based screening was applied analytically (model B), 88.2% of CMV DNA-positive samples were classified correctly. Model B also had a higher sensitivity rate (75%) than that of model A. Based on the current screening protocol demonstrated by model A, no donors were classified as positive in this cohort, so the PPV was undefined, while Model B had a PPV value of 78.9%. On the

other hand, when considering the NPV value of Model A, it is the same value as Model A's overall accuracy - 68.3%. This reflects that the model classified all samples as negative relative to real-time PCR.

Economic Implications of CMV screening classification for milk handling decisions in Poland: The economic simulation estimated downstream cost implications of different screening outcomes, including test costs and milk loss associated with false-positive classifications. Costs were calculated using the minimum, maximum and mean prices of commercially available test kits and the estimated cost per millilitre of donor human milk. The IgM serology test kit ranged from 56.00–75.00 PLN (mean 65.00 PLN), whereas the qualitative PCR kit ranged from 150.00–200.00 PLN (mean 175.00 PLN). Based on data from the Holy Family Hospital in Poland, donor milk volumes ranged from 650 to 32030 ml, with a mean volume of 6481.98 ml (19). The cost per 100 ml of donor human milk in Poland is €7.10 (20), corresponding to €0.07 per ml (0.29 PLN per ml). Accordingly, the estimated cost of milk loss associated with a single positive sample ranged from 188.50 PLN to 1879.77 PLN, depending on donor milk volume. Example calculations and summary cost estimates are shown in Tables 5 and 6.

Model B incurred substantially higher costs than Model A, with minimum estimated costs of 12,581.50 PLN compared with a maximum of 4,500.00 PLN for Model A, illustrated in Figure 4.

Fig 4: Comparison of total policy cost for model A and model B



Overall, the total estimated downstream cost for 20 donors and 60 samples collected at three time points ranged from 3,360.00–4,500.00 PLN for Model A and 12,581.50–47,715.63 PLN for Model B.

Impact for policy makers and clinicians:

Multiple milk samples from the same donor were tested because (a) CMV viral shedding fluctuates with time and (b) milk is collected, processed and stored repeatedly over time as performed in milk banks in day-to-day clinical practice. Therefore, testing multiple milk samples per donor reflects real-world practice and clarifies initial screening assumptions throughout the donation period. Although the analysis focuses on donor human milk findings, they are also relevant to clinical decision-making regarding the usage of a mother's own milk. One of the primary concerns in early perinatal care for preterm infants is the decision regarding the use of pasteurised milk or temporarily withholding the mother's own milk due to the risk of CMV transmission.

Unlike certain milk banks in Sweden, Germany or Switzerland, which use raw unpasteurised human milk and undergo extensive strict screening procedures (1), all milk banks in Poland pasteurise their donor milk using Holder pasteurisation. Holder pasteurisation is a process involving heating milk to high temperatures, followed by cooling it to room temperature to remove noxious pathogens while also preserving bioactive components of breastmilk (6). *This temperature-based technique successfully eliminates the infectivity of pathogens but does not eliminate CMV viral DNA from the BM if they are present in high loads, contributing to increased risk of false positives in saliva amongst infants who were fed this milk during neonatal screening* (21). Such false positives can prompt unnecessary follow-up testing, delay critical care decisions, and potentially undermine confidence in milk-bank safety protocols. Under Model A, no samples were classified as positive, resulting in no milk loss attributable to false-positive classification and overall lower cost estimates. This reflects the high specificity of serology-based screening, whereas Model B classified a higher proportion of the sample as positive (n=19). EMBA recommends routine CMV screening of milk donors because the diagnostic yield for CMV serology is poor. Instead, they recommend proper handling and pasteurisation of DM to eliminate CMV (1,2). An emerging promising technique is high-pressure pasteurisation, which employs 450 MPa for 15 min at 21°C and preserves the immunological properties and natural microbiome of breast milk (22). *Moreover, a recent study from Israel concluded that conducting CMV serology tests among seropositive women, despite guidelines' recommendations, has no clinical value, while it is costly and introduces further uncertainties and distress* (23). The same remains for testing breast milk donors.

Future directions and limitations of the study:

The primary limitation of our study is the small sample size, which limits the stratification of the study population based on gestational age. Larger longitudinal studies are needed to characterise CMV viral DNA shedding patterns

in the breastmilk of donors. The cost estimation of donor human milk per ml based on Polish milk bank data was not recent. The consequent analysis of the total policy estimate may have been undervalued. The Results of our study are consistent with the recommendations of EMBA as they illustrate the classification behaviour and trade-offs of IgM based screening protocol. In addition, we evaluate alternate screening classification protocols, such as qualitative PCR and demonstrate the limited impact on clinical classification for milk banks while substantially increasing the costs of milk handling and processes for milk banks. Future work should focus on developing novel pasteurisation techniques that balance biologic preservation of breastmilk composition and mitigate risk.

CONCLUSION

This study demonstrates that IgM serology-based screening shows poor concordance with CMV viral DNA detection in donor human milk. Compared with IgM serology, qualitative PCR more accurately reflects CMV viral DNA presence but is associated with substantially higher downstream operational costs. Overall, these findings support existing EMBA and HMBANA guidance that routine CMV serological screening of milk donors is not required.

BIBLIOGRAPHY

1. **Weaver G, Bertino E, Gebauer C, et al.** Recommendations for the establishment and operation of human milk banks in Europe: a consensus statement from the European Milk Bank Association (EMBA). *Front Pediatr.*2019;7:53–64.
2. **Shang Z, Li X.** Human cytomegalovirus: pathogenesis, prevention, and treatment. *Mol Biomed.* 2024;5(1):61.
3. **Zając PW, Czarkowska-Pączek B, Wyczałkowska-Tomasik A, et al.** Prevalence and molecular epidemiology of CMV and EBV among nurses working in pediatrics, transplantology, and primary health care. *J Occup Health.*2020;62(1):e12112.
4. **Wesołowska AM.** *Banki mleka w Polsce: idea i praktyka.* Fundacja Bank Mleka Kobiecego, Warsaw, 2017:208.
5. **Johnson KE, Heisel T, Fields DA, et al.** Human cytomegalovirus in breast milk is associated with milk composition, the infant gut microbiome, and infant growth. *Nat Commun.* 2024;15(1):6216.
6. **Singh A, Bartlett A, Clifford V, et al.** Strategies to reduce CMV infectivity in breastmilk to preterm babies: impact on transmission, nutrients, and bioactivity. *J Perinatol.* 2025;45:426–437.
7. **European Directorate for the Quality of Medicines & HealthCare.** *Guide to the quality and safety of tissues and cells for human application.* Council of Europe, Strasbourg, 2019.
8. **Arslanoglu S, Moro GE, Tonetto P, et al.** Recommendations for the establishment and operation of a donor human milk bank. *Nutr Rev.* 2023;81(Suppl 1):1–28.
9. **Sanchez JL, Storch GA.** Multiplex quantitative real-time PCR assay for cytomegalovirus and human DNA. *J Clin Microbiol.* 2002;40(7):2381–2386.
10. **Lagrou K, Bodeus M, Van Ranst M, et al.** Evaluation of the Architect cytomegalovirus IgM, IgG, and IgG avidity assays. *J Clin Microbiol.* 2009;47(6):1695–1701.
11. **Chiavarini M, Bragetti P, Sensini A, et al.** Breastfeeding and transmission of cytomegalovirus to preterm infants. *Ital J Pediatr.* 2011;37:6.
12. **Park HW, Cho MH, Bae SH, et al.** Incidence of postnatal CMV infection among breastfed preterm infants: a systematic review and meta-analysis. *J Korean Med Sci.* 2021;36(12):e84.

13. **Loughnan M, Mehring-Le-Doare K.** Breast milk composition. In: Shackelford T, Weekes-Shackelford V, eds. *Encyclopedia of Evolutionary Psychological Science.* Springer, Cham, 2020.
14. **Azenkot T, Zaniello B, Green ML, et al.** Cytomegalovirus shedding from breast milk and mucosal sites in healthy postpartum women. *J Med Virol.* 2019;91(5):894–898.
15. **Lanzieri TM, Dollard SC, Josephson CD, et al.** Breast milk-acquired cytomegalovirus infection in very low birth weight and premature infants. *Pediatrics.* 2013;131(6):e1937–e1945.
16. **Huang T, Cai W, Ni C, et al.** Changes in cytomegalovirus load in breast milk of very premature infants and effects of pasteurization and freeze-thawing. *Front Pediatr.* 2022;10:900249.
17. **Rabe T, Lazar K, Cambronero C, et al.** Human cytomegalovirus reactivation in the mammary gland induces a proinflammatory cytokine shift in breast milk. *Microorganisms.* 2020;8:289.
18. **Lazar K, Rabe T, Goelz R, et al.** Human cytomegalovirus reactivation during lactation: impact of antibody kinetics and neutralization in blood and breast milk. *Nutrients.* 2020;12:338.
19. Barbarska O, Zielińska M, Pawlus B, Wesołowska A. Characteristics of the regional human milk bank in Poland - donors, recipients and nutritional value of human milk. Rocz Panstw Zakl Hig. 2017;68(4):395-400. PMID: 29280369.
20. **Wesołowska A, Dobrowolska I, Sinkiewicz-Darol E, et al.** Donor human milk for preterm infants: a cost-consequence analysis of alternative preservation methods. *Value Health Reg Issues.* 2020;22:54–60.
21. **Wunderlich W, Sidebottom AC, Schulte AK, et al.** Saliva testing for congenital cytomegalovirus infection and false-positive results associated with donor milk use. *Int J Neonatal Screen.* 2023;9(3):46.
22. **Jarzynka S, Strom K, Barbarska O, et al.** High-pressure processing combined with freeze-drying preserves biological value and microbiological safety of donor milk. *Int J Environ Res Public Health.* 2021;18(4):2147.
23. **Angel Y, Fire G.** Healthcare policy changes in an era of health workforce shortage. *Isr J Health Policy Res.*2023;12(1):28

TABLES

Table 1: Definition of outcome classification [TP, FP, FN, TN] for serology-based and qualitative PCR

Analysis metrics	Classification for metric
True positives [TP]	Test results are positive, and real-time PCR shows viral shedding (> 0.3 CMV viral DNA copies/µl).
False positives [FP]	Test results are positive, and real-time PCR shows no viral shedding (real-time PCR reads negative).
True negatives [TN]	Test results are negative, and real-time PCR shows no viral shedding (real-time PCR reads negative).
False negatives [FN]	Test results are negative, and real-time PCR shows viral shedding (>0.3 CMV viral DNA copies/µl).

Test results: refers to either IgM based serology model or the qualitative PCR model

Table 2: calculations used to assess the diagnostic accuracy of models

Diagnostic accuracy metrics	Calculation method
Sensitivity	TP / (TP + FN)
Specificity	TN / (TN + FP)
PPV (Precision)	TP / (TP + FP)
NPV	TN / (TN + FN)
Accuracy	(TP + TN) / (TP + FP + FN + TN)
Exclusion Rate	(TP + FP) / Total samples

caption: values for TP, FP, FN, TN were obtained using the criteria set in Table 1. These references are obtained from published material by Wong.H.B et.al [1]

TABLE 3: Raw counts of model A vs model B in the

Diagnostic metric N= counts	Model A overall	Model B overall
True positive	0	15
True negative	41	39
False negative	19	2
False positive	0	4
sum	60	60

TABLE 4: DIAGNOSTIC PERFORMANCE METRICS OF IgM SCREENING MODEL vs QUALITATIVE MODEL.Note: 95% confidence intervals adjusted to our sample size using

[1] 1.
Wong HB, Lim GH. Measures of Diagnostic Accuracy: Sensitivity, Specificity, PPV and NPV. *Proceedings of Singapore Healthcare*. 2011;20(4):316-318. doi:10.1177/201010581102000411

Diagnostic accuracy metrics	IgM serology model (model A)	Qualitative PCR model (model B)
Sensitivity (%)	0 (0.00–16.8)	88.2 (65.7–96.7)
Specificity(%)	100 (91.4–100.0)	90.7 (78.4–96.3)
Positive predictive value (PPV%)	N/A	78.9 (56.7–91.5)
Negative Predictive value (NPV%)	68.3 (55.8–78.7)	95.1 (83.9–98.7)
Accuracy (%)	68.3 (55.8–78.7)	90.0 (79.9–95.3)
Exclusion rate (%)	0 (0.00–16.8)	31.7 (21.3-44.2)

Wilson's coefficients are depicted within parentheses() where appropriate

TABLE 5: Policy simulation calculation with example

*Total policy cost [model A/model B]= (minimum/average/maximum test cost * 60 samples) + cost of milk loss (minimum/maximum cost of donor human milk * number of false positives+ true positives from model A/model B).*

Example calculation:

Model B total Policy cost for “minimum” estimate = (150.00*60)+ (182.70*19)

= 12471.30 PLN

TABLE 6: Cost estimation of model A vs model B

Model type	Test cost per donor [PLN]	Total test cost for 60 samples [PLN]	Minimum milk loss cost [PLN]	Maximum milk loss cost [PLN]	Total policy cost [PLN]
Model A IgM screening N=60 samples, exclusion rate=0%	Low: 56.00	3360.00	0.00	0.00	3360.00
	Mean: 65.50	3930.00	0.00	0.00	3930.00
	High: 75.00	4500.00	0.00	0.00	4500.00
Model B Qualitative PCR N= 60 samples, Exclusion rate=31.6% [TP=15,FP=4]	Low: 150.00	9000.00	188.50*19	1879.77*19	Min:12581.50 Max: 44715.63
	Mean: 175.00	10500.00	188.50*19	1879.77*19	Min:14081.50 Max: 46215.63
	High: 200.00	12000.00	188.50*19	1879.77*19	Min: 15581.50 Max: 47715.63

FIGURE LEGEND

FIGURE LEGENDS WITH CORRESPONDING TITLE AND DESCRIPTION

Figure 1:Interindividual variability of CMV viral DNA shedding in lactating mothers

Legend: only 6 donors are represented, and they constituted the 35% of the population that were shedding CMV viral DNA in breastmilk as identified by real-time PCR [n=6]. Mother_6 and mother_2 are the only ones showing an increase in viral load beyond Time_2. They were not united by donor demogrpahich denominataors like gestational age or lactation stage depicting heterogentiy of CMV viral kinetics in breastmilk.

Figure 2: Mean CMV viral load over time to highlight temporal shedding trends

Legend: Corresponding Standard Error of the Mean [SEM] was used to validate the credibility of the mean VL depicted as error bars in Figure 2. Notably, a larger Standard Error value for Time_1 compared to the other time points suggests greater inter-individual variability in viral shedding amongst donors at baseline.

FIGURE 3: Forest plot representing epidemiological metric as point estimates with 95% Wilson confidence intervals for the overall performance of Model A vs Model B

Legend: The vertical dashed line at 0.5 serves as an indicator to distinguish the activity of Model A in blue and model B in green, representing IgM-based serology screening and Qualitative PCR, respectively.

FIGURE 4: COMPARISON OF TOTAL POLICY COST FOR MODEL A and MODEL B

Legend: In the graph, the test cost and total policy cost with milk loss costs are the same in Model A, distinctly visible by the absence of the secondary area plot as seen in Model B. However, when using qualitative PCR the milk loss cost is significant

Overall, the area under Model B is greater, depicting the higher operational costs of adopting a different screening tool.

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Competing Interests

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Ethics approval

All procedures in this study were conducted in accordance with the Declaration of Helsinki. Donor recruitment and human milk donation procedures were carried out in compliance with the European Milk Bank Association guidelines for good practice, maintaining the highest standards of scientific integrity and research quality.

Consent to participate

Written informed consent for milk donation included explicit consent for the use of surplus biological material and laboratory data for research purposes aimed at improving human milk bank operational procedure and safety standards. All samples were coded prior to analysis to ensure donor confidentiality and **data protection**