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Skimmed Milk Flocculation Technique for Waste Water

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Coronavirus Method Development Community

SARS Cov2 ES



Venkata Raghava Mohan

ABSTRACT

The direct examination of waste water is difficult due to low and fluctuating concentrations of microorganisms and because concentration procedures are usually organism and/or matrix specific and most techniques have high or unknown variability parameters. One-step skimmed milk flocculation (SMF) has been proposed as an efficient method to concentrate viruses in all types of environmental water matrices such as river water, seawater, groundwater and wastewater. The organic flocculation procedure is easy and fast to be carried out; it requires a simple laboratory infrastructure, thus eliminating costly equipment, in addition to the low cost of the inputs necessary to be performed. The aim of this study is to perform skimmed milk flocculation procedure in order to detect SARS-CoV-2 recovery from the waste water.

ATTACHMENTS

[Skimmed Milk flocculation protocol_CMC_India.pdf](#)

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KEYWORDS

Skimmed milk flocculation, SARS-CoV-2, Waste water

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GUIDELINES

Principle and methods of the procedure used for examination:

Organic flocculation is a quick, easy, time-saving and inexpensive method used to concentrate virus and remove impurities from waste water without ultracentrifugation. The technique entails the adsorption of viruses to pre-flocculated skimmed milk proteins, allowing the flocs to sediment by gravity, and dissolving the separated sediment in phosphate buffer (PBS).

Sample collection- Type of sample:

Waste water

MATERIALS TEXT

Required Consumables:

[ART Barrier Pipet Tip, 1000, soft fit, sterile Thermo](#)

Fisher Catalog #2279

[ART™ Barrier Pipette Tips in Lift-off Lid Rack Thermo](#)

[20 µl - 200 µl Fisher Catalog #2069](#)

[Schott Duran 21801545 Glass Graduated Media Storage Bottle Cap Round 1000ML GL-45 Lab](#)

glassware Catalog #21801545

[Nunc™ Conical Sterile Polypropylene Centrifuge Tubes, 15mL, bulk Thermo](#)

Fisher Catalog #339650

[50 ML Centrifuge](#)

[Tubes P'fact Catalog #PCT-50-B-S](#)

Waste water

A	B	C
Name	Company	Catalogue No.
100 – 1000 µl	ART	2279
20-200 ul Pipette and tips	ART	2069
Eppendorf micro-centrifuge tubes Safe Lock	Axygen	MCT175C
Lab coat	MP	6037
Disposable non-sterile gloves	Hi care	327031
1000 ml bottle	Schott Duran	21801545
50 ml serological pipette	Tarsons	
10 ml serological pipette	Tarsons	NA
5 ml serological pipette	Genaxy	326001
15 ml centrifuge tube	Thermo Scientific	339650
50 ml centrifuge tube	PFact	PCT 50bs

Required Reagents:

[Skim Milk Becton-](#)

Dickinson Catalog #232100

[Phosphate Buffered Saline \(PBS\) tablets MP](#)

Biomedicals Catalog #0928103-CF

A	B	C
Name	Company	Catalogue No.
Skim milk	BD	232100
Hydrochloric acid	Fisher Scientific	29505
Sodium Hydroxide	Merck	60995600011730
Phosphate-Buffer Saline	MP Biomedicals	092810305
Absolute ethanol (100%)	Hayman SpecialityProducts/ Honeywell	F203640/ 3221
10% Sodium Hypochlorite	S10673	Nice
1% Sodium Hypochlorite	S10673	Nice

Required Equipments:

 SPINIX™ – Vortex

Shaker Tarsons Catalog #3004

 Deep Freezers Cryoscientific

systems Catalog #URC-V-570-1



 Eppendorf® Centrifuge 5910 R G Sigma




Aldrich Catalog #EP5942000010

A	B	C
Name	Company	Catalogue No.
Refrigerators	Godrej Ultra	NA
Vortex mixer	Spinix	3004
Biological Safety Cabinet	Kartos international	1001-B
Ultracold Freezer	Cryo-scientific -20°C	URC-V-570-1
MegaCentrifuge	Eppendorf	5910-RG

Reagent Preparation

a. Preparation of 5% pre-flocculated Skim Milk:

a.i To make 5% pre-flocculated skim milk solution (w/v), dissolve  5 g skimmed milk powder in  100 mL MilliQ water.


a.ii Autoclave for  00:15:00 at  115 °C , 18 psi and allow it to cool at the  Room temperature .








a.iii The prepared skim milk solution can be stored at  4 °C for up to  72:00:00 .

Skimmed Milk Flocculation




2h 45m

1 For virus concentration assay, collect a total of  250 mL waste water, and divide into two aliquots ( 100 mL).

2 




To each  100 mL sample, add  1 mL 5% pre-flocculated skim milk (e.g., add  2.5 mL for  250 mL of sample) and adjust the  pH3.5 -  pH4.0 by the addition of  1 Molarity (M) HCl.

3  

Place the samples on a horizontal shaker and incubate in agitation at  **200 rpm** for  **02:00:00** at  **Room temperature** to allow the floc formation.

4 

30m





After incubation, allow the flocculants to sediment by centrifugation at  **3500 x g** for  **00:30:00** at  **4 °C** using a swinging bucket rotor.


5 Remove the supernatant carefully from the tube without disturbing the bottom and suspend the pelleted virus with the following buffer.

6 Use 1 set of pellet for RNA extraction using the QIAamp viral RNA mini kit by suspending it with the lysis buffer provided in the kit.

7 

15m

Dissolve another aliquot in  **1.5 mL** of sterile 1x PBS ( **pH7.4**) and vortex both the suspension for  **00:05:00** -  **00:10:00** at maximum speed to completely dissolve the pellet.

8 Store the final viral concentrates at -  **80 °C** until further processing.