Enrichment of Biomolecules

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iST Innuscreen GmbH aus Berlin

Why target enrichment ?

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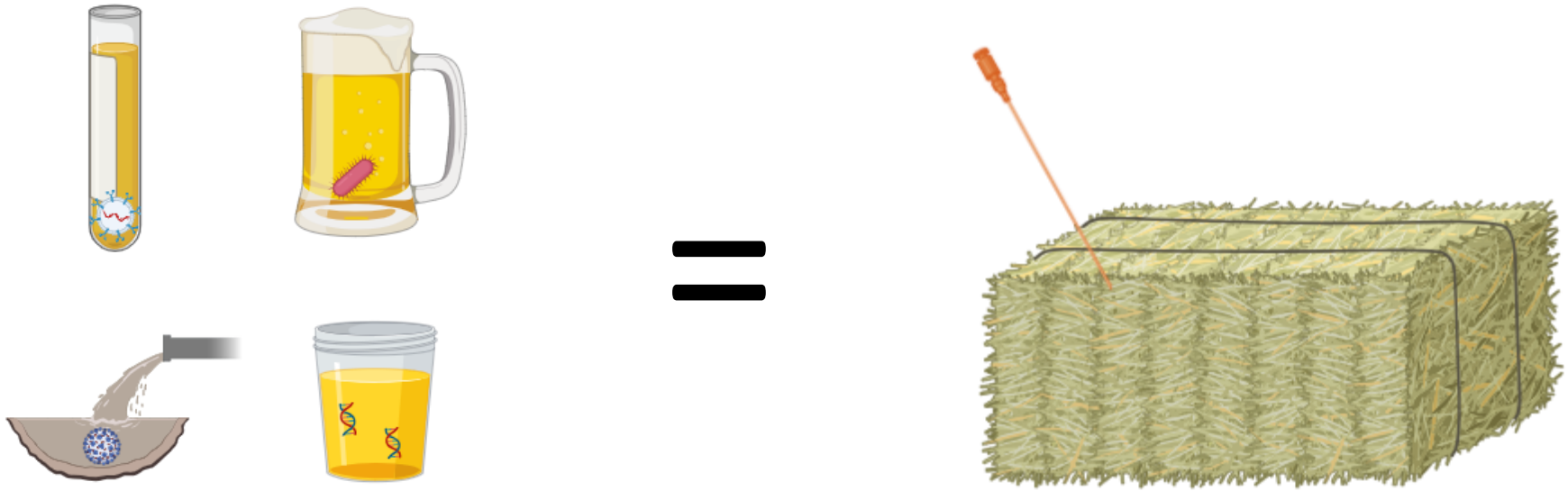

large sample volume + few target molecules = needle in the haystack

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Why target enrichment ?

Innuscreen GmbH

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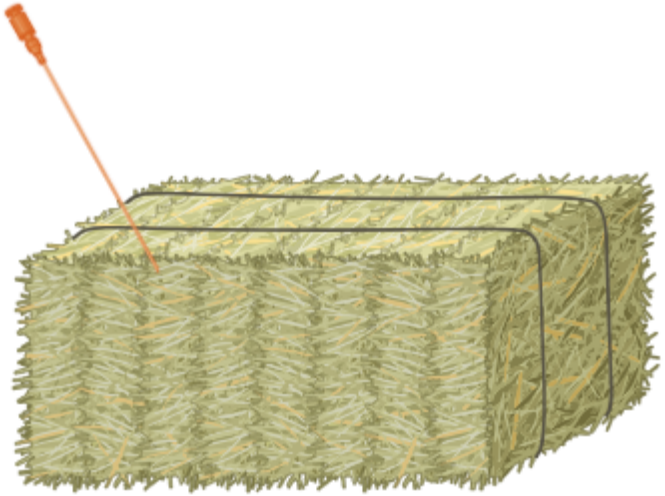
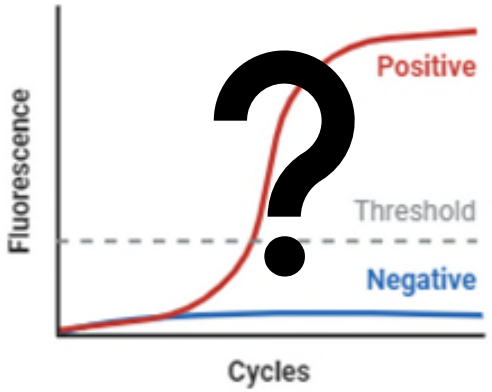


large sample volume + few target molecules = needle in the haystack

direct DNA/RNA extraction + PCR often not possible!



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What are the targets of interest ?

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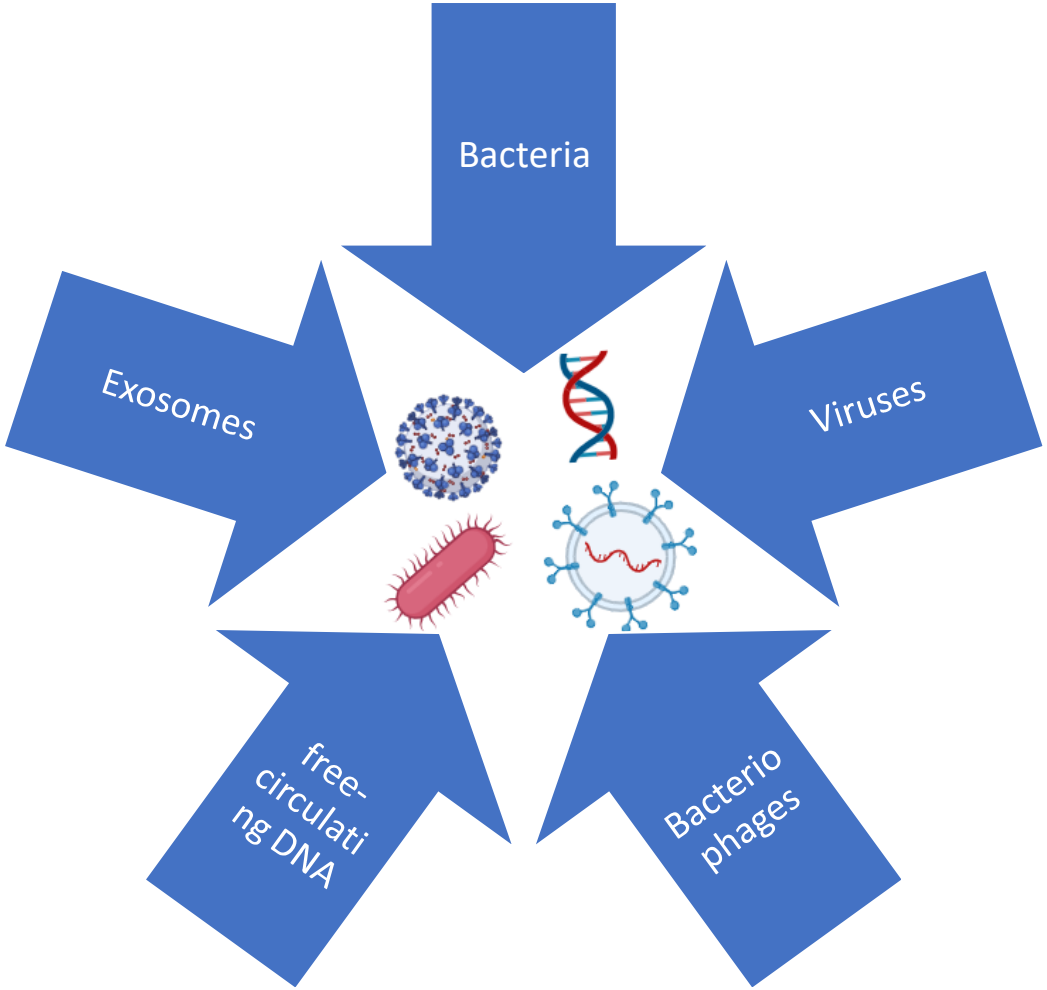

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What is the challenge?

Innuscreen GmbH

Target enrichment necessary, but sometimes challenging!

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Innuscreen GmbH

Target enrichment necessary, but sometimes challenging!

Centrifugation?

- good for bacteria, but ultra-centrifugation necessary for viruses, cell-free DNA, exosomes…
- difficult for high sample volumes

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What is the challenge?

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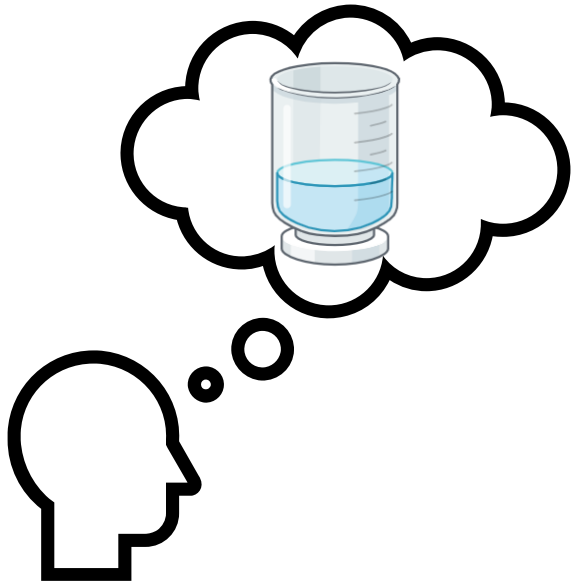
Target enrichment necessary, but sometimes challenging!



Filtration?

- risk of filter clogging
- sample loss if pore size too large

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What is the challenge?

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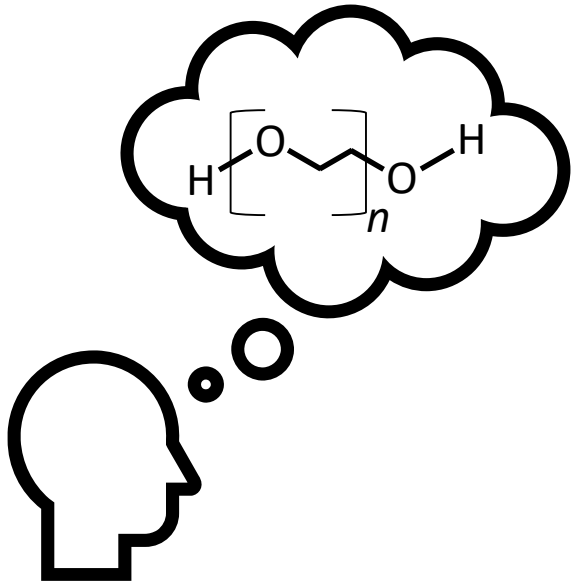
Target enrichment necessary, but sometimes challenging!



PEG Precipitation?

- time-consuming
- labor-intensive

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New enrichment technologies by IST Innuscreen

Our Solution:

Two new technologies for enrichment of biomolecules for sample pre-treatment of aqueous liquids (water, beverages, body fluids).

PME -
Polymer
Mediated
Enrichment

TCT –
Target
Concentration
Technology

PME Technology

Innuscreen GmbH

General principle of PME

Sample + Reagent 1 and 2 – formation of biomolecule/polymer-complex

Centrifugation of biomolecule/polymer-complex

Dissolving of biomolecule/polymer-complex (e.g. in lysis buffer for DNA isolation)

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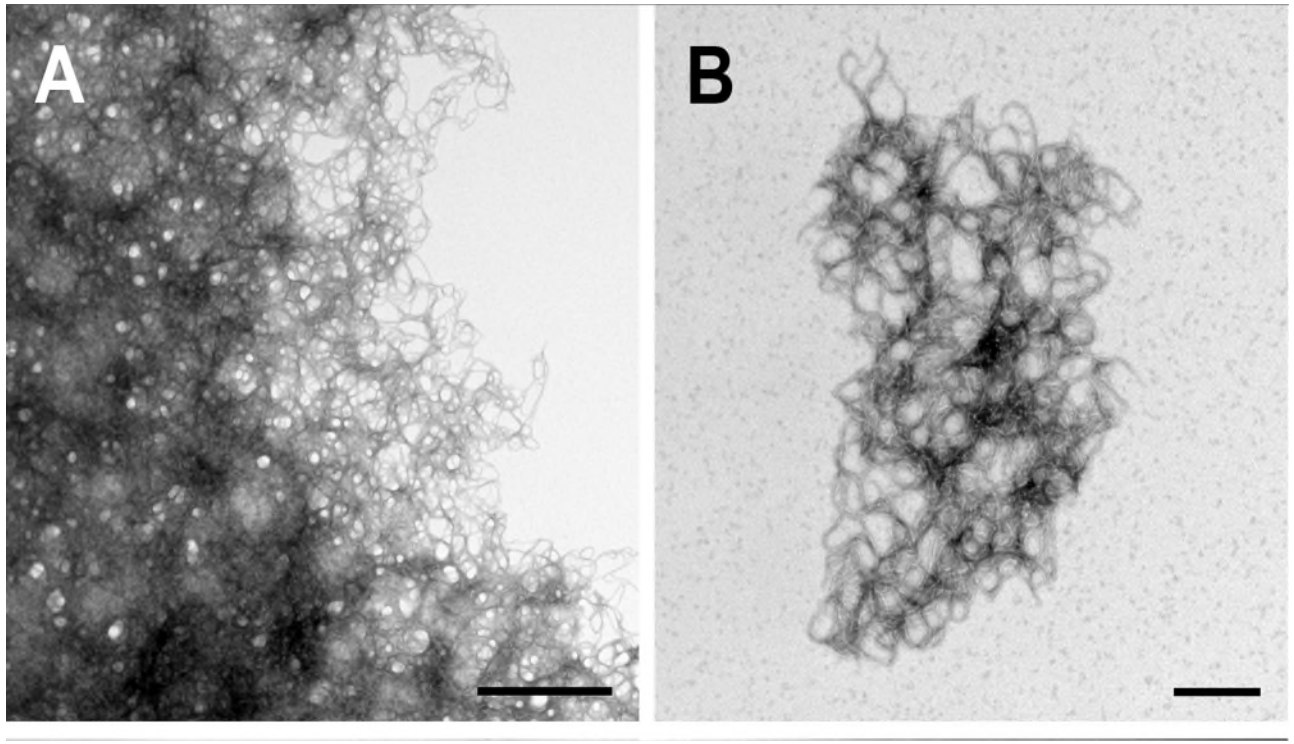
PME Technology

Innuscreen GmbH

Polymer Mediated Enrichment (PME) – Enrichment of virus particle



Electron micrograph of polymer-structure

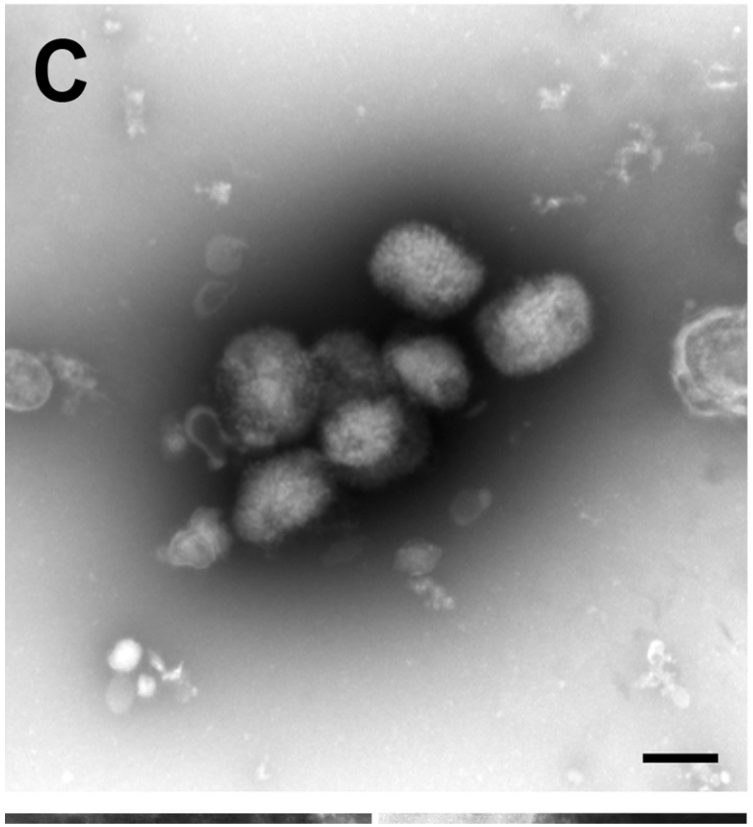
A-B: Electron micrograph of polymer complex;
Bars: A = 500 nm, B = 200 nm

(data kindly provided by P. Patel; RKI Berlin)

PME Technology

Innuscreen GmbH

Polymer Mediated Enrichment (PME) – Enrichment of virus particle



Electron micrograph of Camelpox Virus

C: Electron micrograph of Camelpox Virus

Bars: C = 200 nm,
D = 200 nm

(data kindly provided by P. Patel; RKI Berlin)

PME Technology

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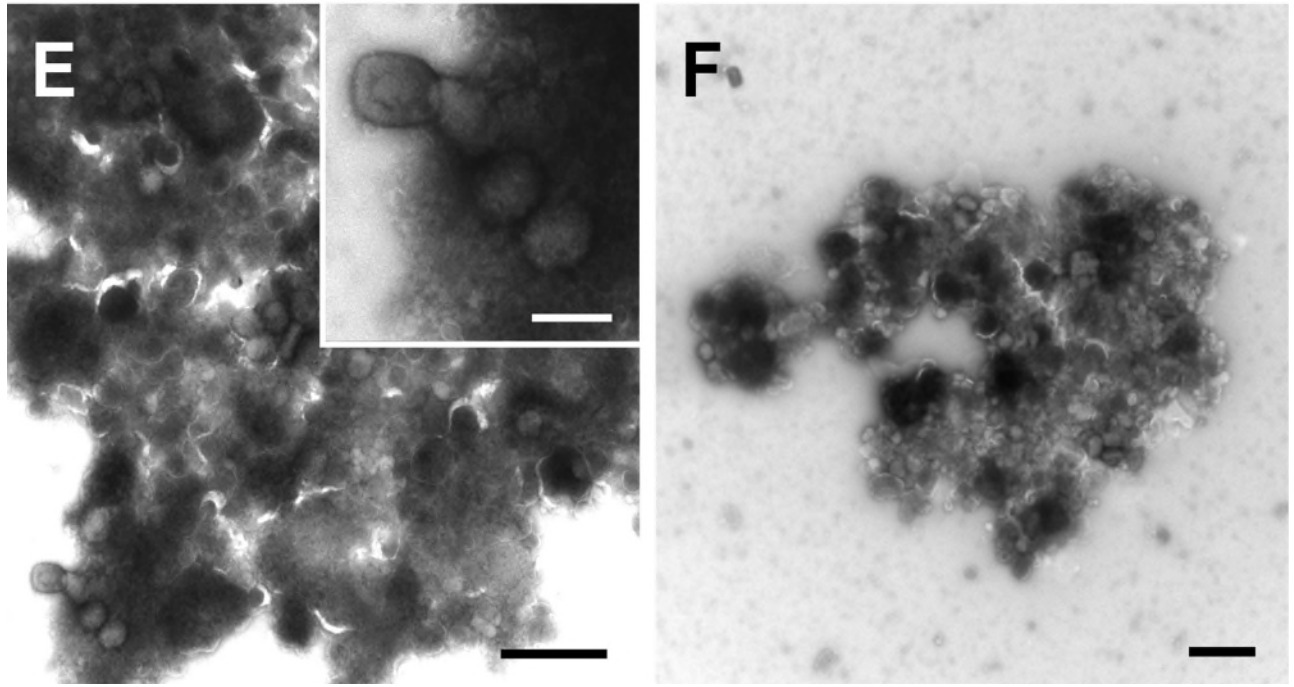
Polymer Mediated Enrichment (PME) – Enrichment of virus particle



Electron micrograph of polymer- Camelpox virus

E-F: Electron micrograph of polymer-virus complex after enrichment
Bars: E = 1 µm
F = 1 µm

(data kindly provided by P. Patel; RKI Berlin)

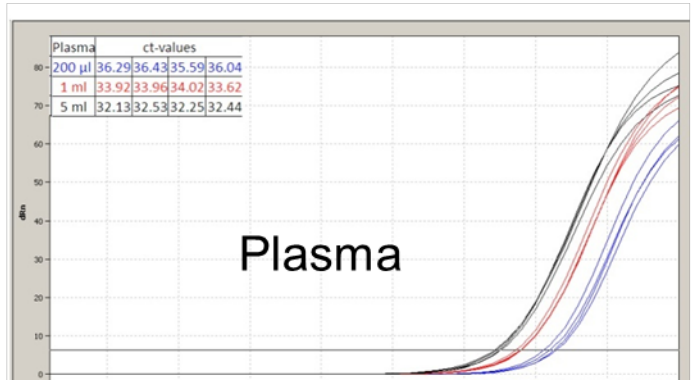
PME Technology

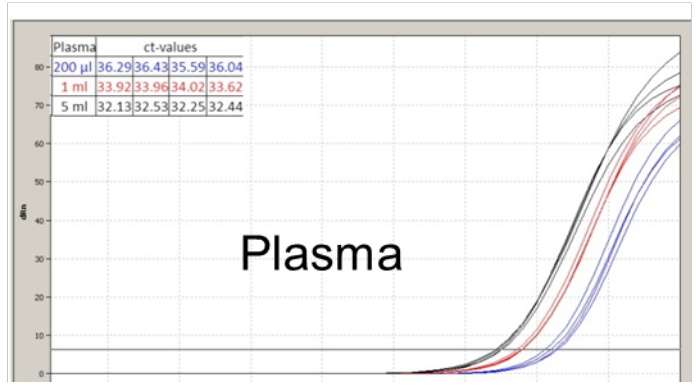
Innuscreen GmbH

Sometimes more is more….

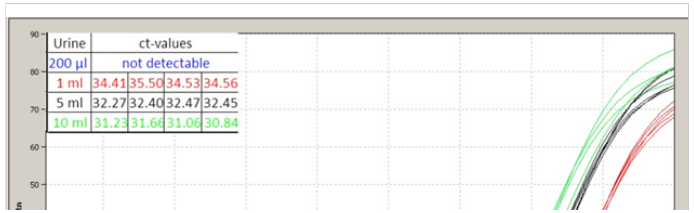
Extraction of cell-free DNA followed by qPCR detection (human-specific sequence) with

- **200 µl (standard spin filter extraction)**
- **1 ml (enriched using PME Technology)**
- **5 ml (enriched using PME Technology)**
- **10 ml (enriched using PME Technology)**





Higher DNA yield by higher sample v



PME Technology

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Summary PME Technology

- suitable for water, cell culture supernatant, serum, plasma, liquor, urine...
- for sample volumes of up to 50 ml
- enrichment of viruses, virus fragments, free-circulating DNA, bacteriophages, bacteria, exosomes
- enriched viruses suitable for re-cultivation, cell assays etc. (no harmful effects by polymer)

Target Concentration Technology (TCT)

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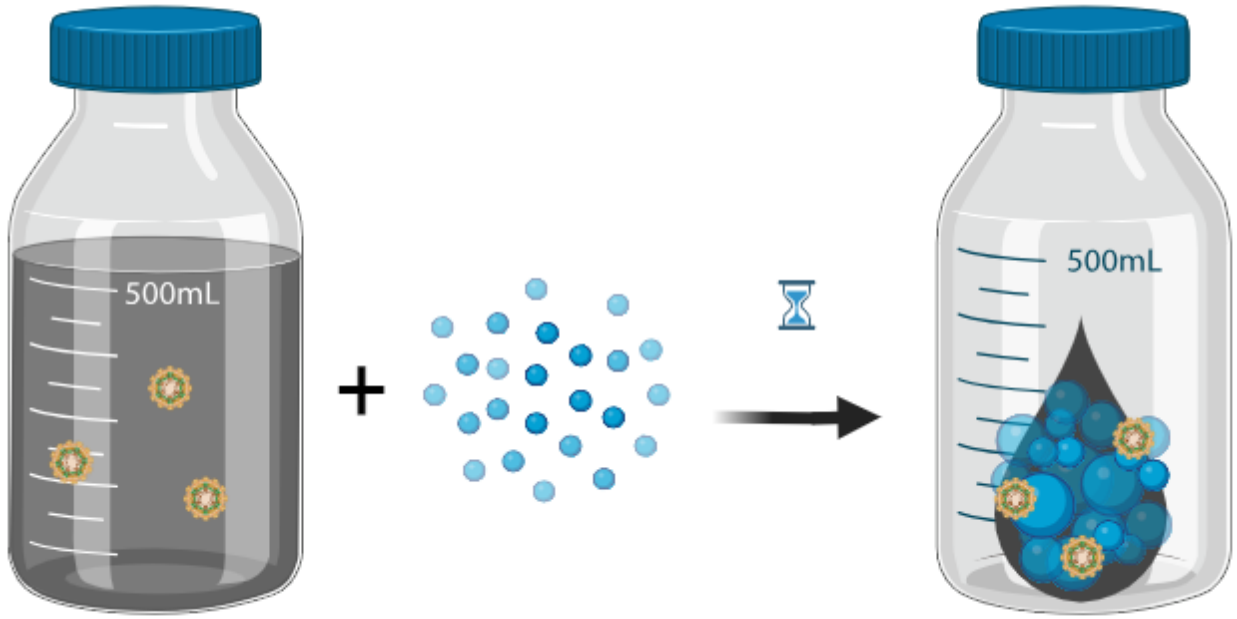
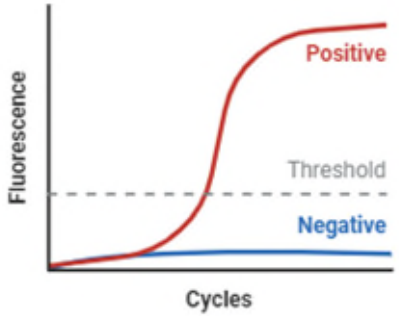
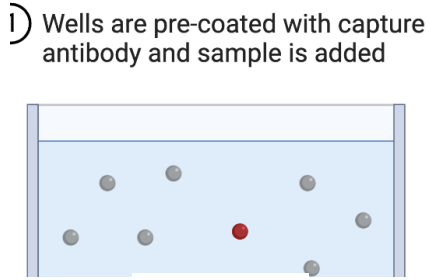
General Principle of TCT

Sample (up to 1000 ml) + TC Beads → volume reduction





qPCR



ELISA

Western Blot

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Target Concentration Technology (TCT)

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Example: concentration of bacteriophages in water samples

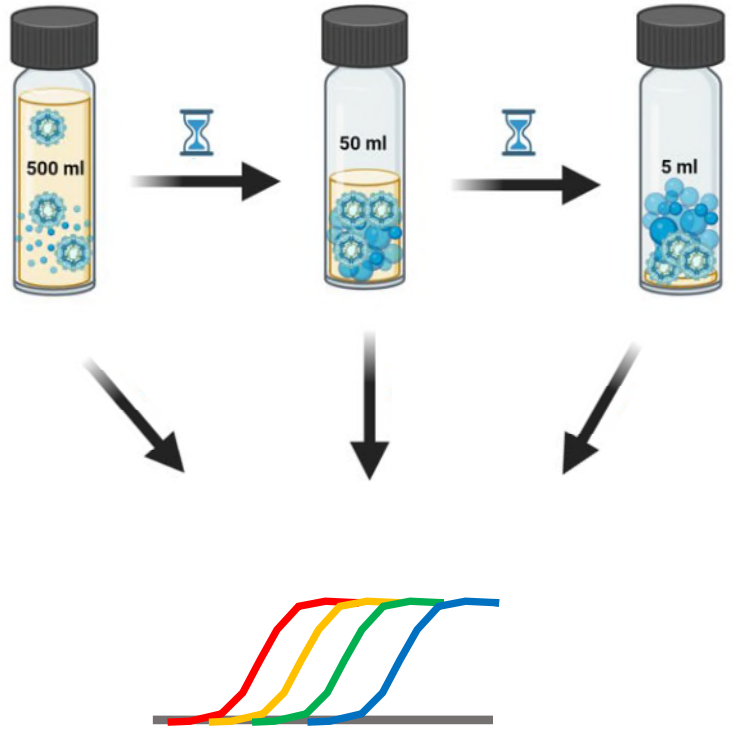
Sample: 500 ml drinking water

Spike: bacteriophage MS2

Procedure:
addition of TCT beads to the sample and
target enrichment in 2 steps:

Step 1 – volume reduction to 50 ml
Step 2 – volume reduction to 5 ml

Extraction of MS2-RNA from 200 µl of each sample
and subsequent RT-PCR for detection



qPCR

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Target Concentration Technology (TCT)

Innuscreen GmbH

Concentration of bacteriophages in water samples

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Step 2 – volume reduction to 5 ml (△)

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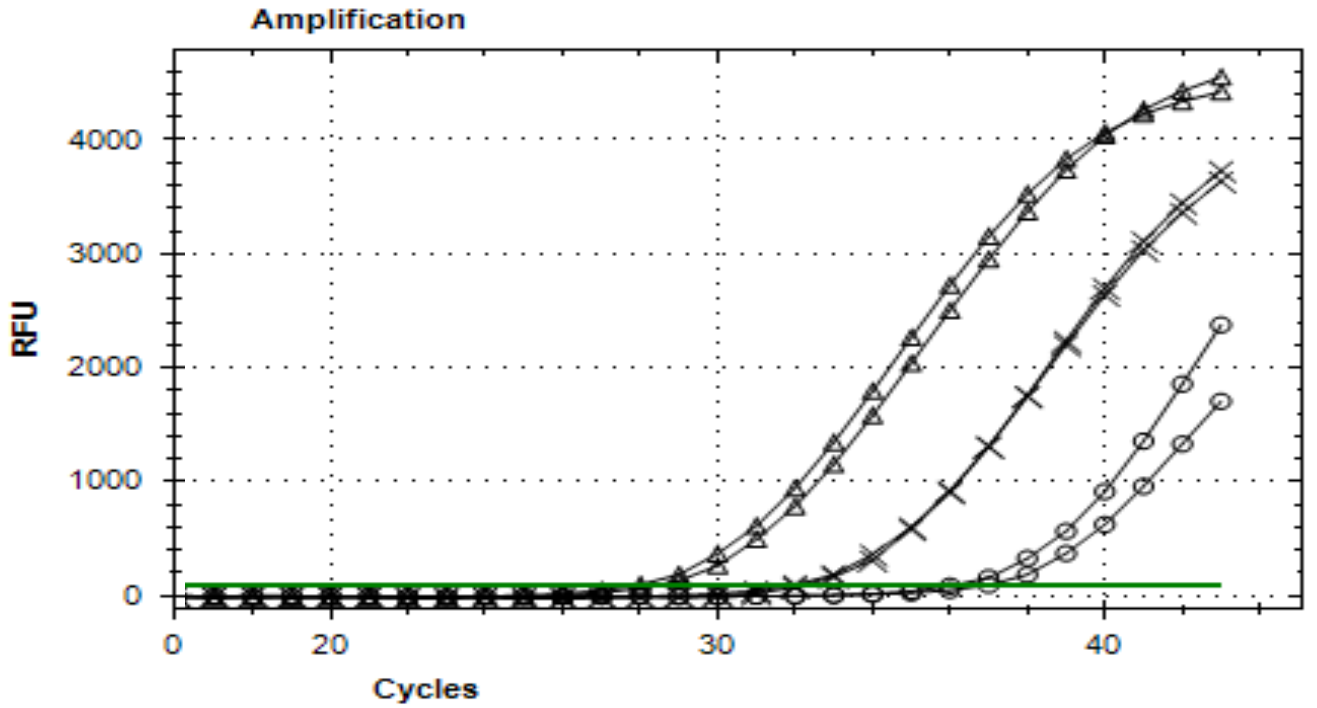


sample	symbol	CT value	enhancing factor
initial sample 1 (200 µl of 500 ml)	○	35,93	0
initial sample 2 (200 µl of 500 ml)	○	36,73	0
sample 1 from 1. enhancing step (200 µl of 50 ml)	X	31,72	10
sample 2 from 1. enhancing step (200 µl of 50 ml)	X	32,07	10
sample 1 from 2. enhancing step (200 µl of 5 ml)	△	28,23	100
sample 2 from 2. enhancing step (200 µl of 5 ml)	△	27,68	100

Target Concentration Technology (TCT)

Innuscreen GmbH

Detection of low concentrations of free circulating plasmid DNA

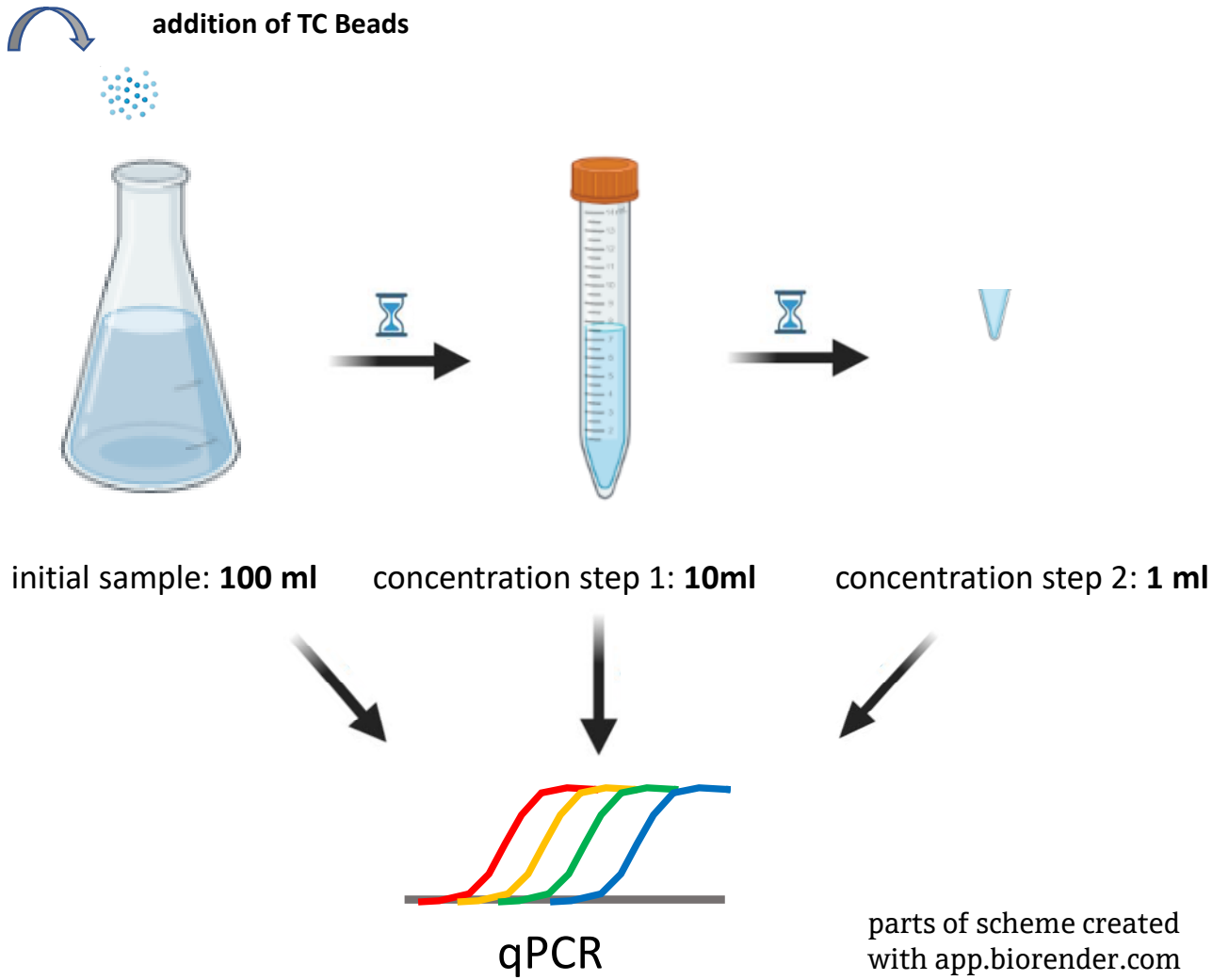
Sample: 100 ml drinking water

Spike: 100 fg plasmid DNA (1 fg/ml = 580 molecules/ml)

Procedure:
addition of TCT beads to the sample and
target enrichment in 2 steps:

Step 1 – volume reduction to 10 ml
Step 2 – volume reduction to 1 ml

Extraction of plasmid DNA
and subsequent RT-PCR for detection



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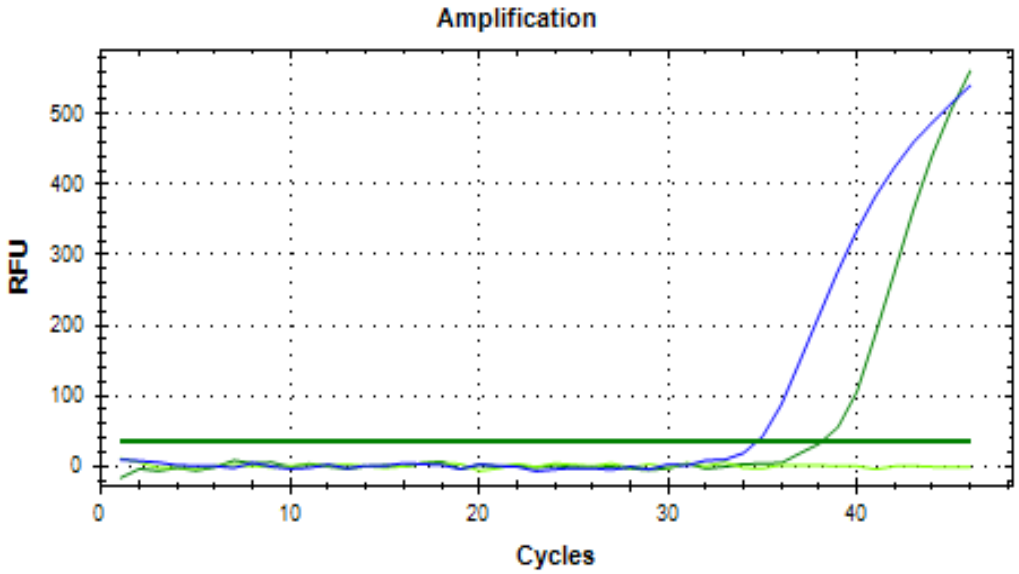

Sample	color	Ct value	volume	concentration factor
1	light green	no detection	100 ml	
2	green	38,1	10 ml	10
3	blue	34,8	1 ml	100

Target Concentration Technology (TCT)

Innuscreen GmbH

Enrichment of bacteria from a bacterial culture

Sample: E.coli

Samples volume: 50 ml growth medium

Procedure:
addition of TCT Beads to 50 ml of bacterial culture and concentration from 50 ml to 10 ml

Plating out on agar plates and incubation

Target Concentration Technology (TCT)

Innuscreen GmbH

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Sample: E.coli

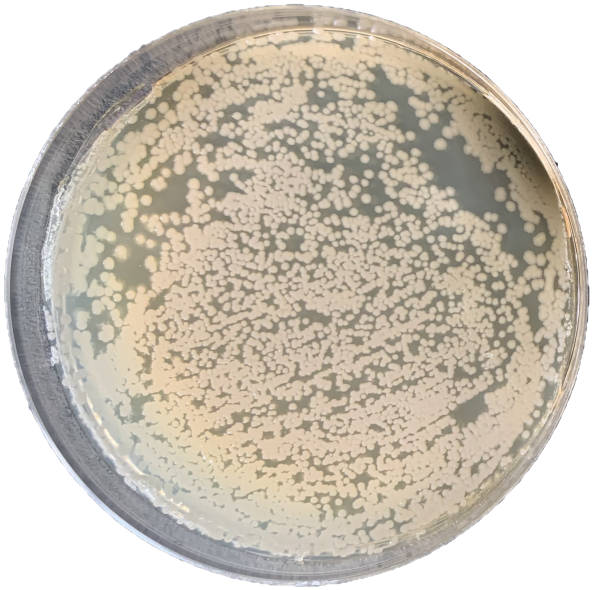
Samples volume: 50 ml growth medium

Procedure:
addition of TCT Beads to 50 ml of bacterial culture and concentration from 50 ml to 10 ml

Plating out on agar plates and incubation

no enrichment

with enrichment (factor 5)



Target Concentration Technology (TCT)

Innuscreen GmbH

Summary TCT

- for sample volumes of up to 1000 ml
- for enrichment of viruses, virus fragments, free-circulating NA, bacteriophages, bacteria, proteins, etc.
- enriched sample can be directly used for downstream applications
- compatible with any kind of DNA/RNA extraction method
- enrichment of living bacteria possible

Thank you for your attention!