

Nucleic Acid Isolation for Diagnostic Testing using Bayer's Magnetic Particles

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Diagnostics

Bayer HealthCare Divisions



Diagnostics

- Laboratory Testing
- Near Patient Testing
- Molecular Testing**



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Pre-Analytical Steps: Sample Preparation

- Efficient isolation of nucleic acids is a key prerequisite for their qualitative and quantitative detection in diagnostic applications

- ◆ Pure nucleic acids / Interference free

- ◆ Process automation

- ◆ Sensitive with high recovery/purification efficiency

Clinical
Need for
Technical
Performance

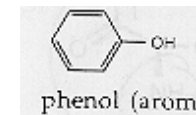


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History of Nucleic Acid Isolation

- Phenol / Chloroform

- Special case



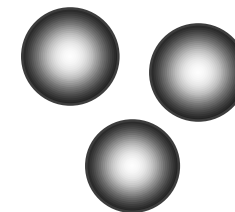
- Column Technology (Silica particles)

- Research (automation difficult)



- Silica-coated magnetic particles

- In Vitro Diagnostic



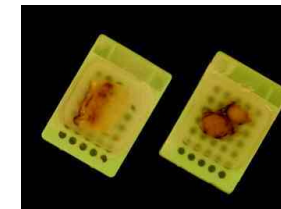
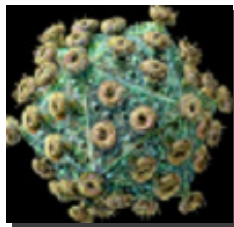
Innovation



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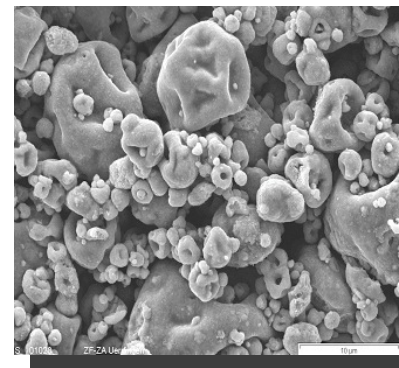
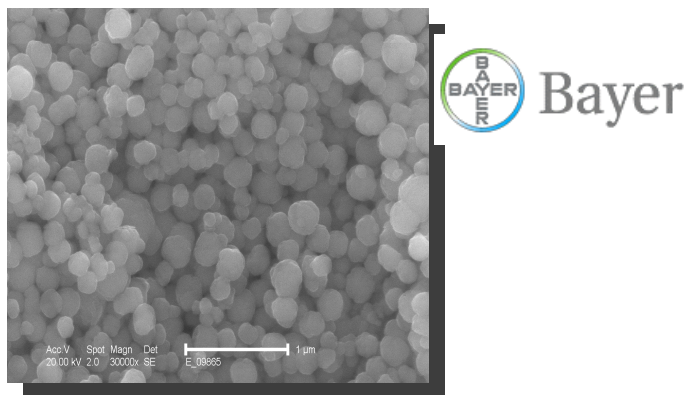
Advantages of Magnetic Particle Technology

- Simple and low-cost automation
- High reproducibility
- High sample throughput
- Scalability of sample volume
- Flexible in its applications



Bayers „new“ Magnetic Particles

- „One particle chemistry“ for all applications in the field of nucleic acid isolation
- Coating of iron oxides with nanolayer of silica
- Simple, robust and controlled manufacturing process
- No change in morphology



Others

Properties Determine Performance

Properties

- „homogenous“ particle size distribution
- small particles ($< 1 \mu\text{m}$)
- very high iron content
- very good paramagnetism
- high storage stability

Automation Advantage

- Reproducibility
- optimal suspension behaviour
- efficient magnetization
- efficient isolation
- Reliability

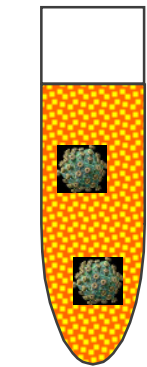
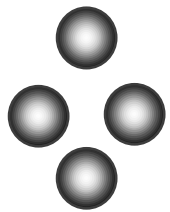
Infectious Disease Testing Assay Portfolio

Kinetic Amplification Assays in Development

- HCV (RNA) currently the assay has a sensitivity of 10 copies/reaction (genotypes 1 through 6)
- HIV (RNA) current level of sensitivity is 10 copies/reaction and (subtypes of group M and group O)
- HBV (DNA) current level of sensitivity is 10 copies/reaction
- CT/GC (DNA) current level of sensitivity is 10 copies/reaction

Schematic Nucleic Acid Isolation Protocol

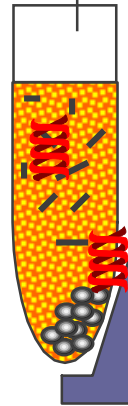
Chaotropic binding buffer
Proteinase K
Magnetic particle



Plasma

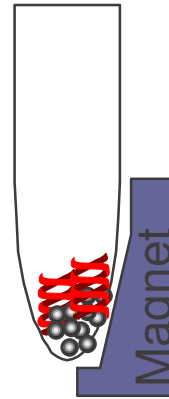
Mix
Incubation

Supernatant

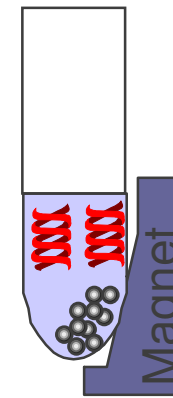


Binding

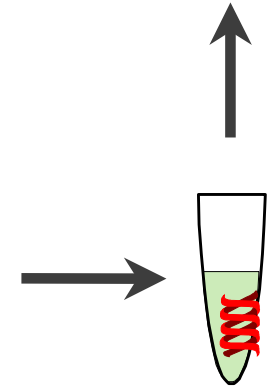
Wash



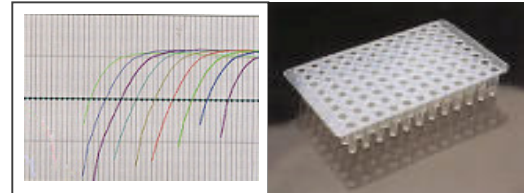
Elution



Release



Eluate +
MasterMix



Detection and Quantification
with kinetic Amplification

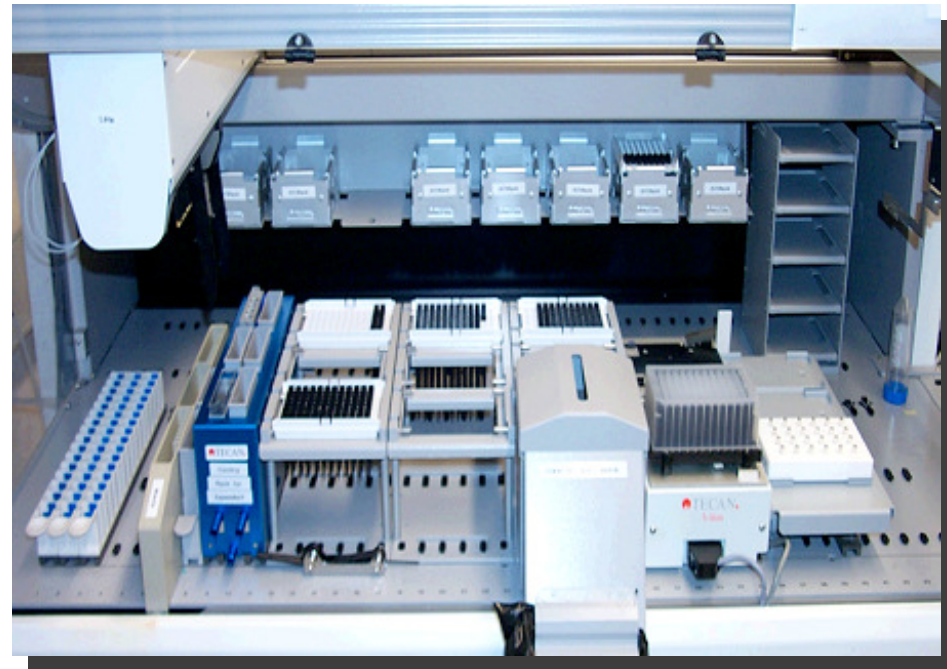
Automation of HCV Isolation (Model System)

- Commercial Liquid Handling Robot

- Module

- ◆ Samples
- ◆ Reagents
- ◆ Tips
- ◆ Waste
- ◆ Microtiter plate
- ◆ Shaker
- ◆ Heating block
- ◆ Magnet

- Complete automation



Set-Up Flexibility

- Automated protocol allows flexible purification of different sample batches
8, 16, 24 ... - 96 samples (< 2,5 hours for 96)
- Automated protocol allows flexible purification of different sample volumes
500 μ l or 1 ml

HCV Samples for Performance Study

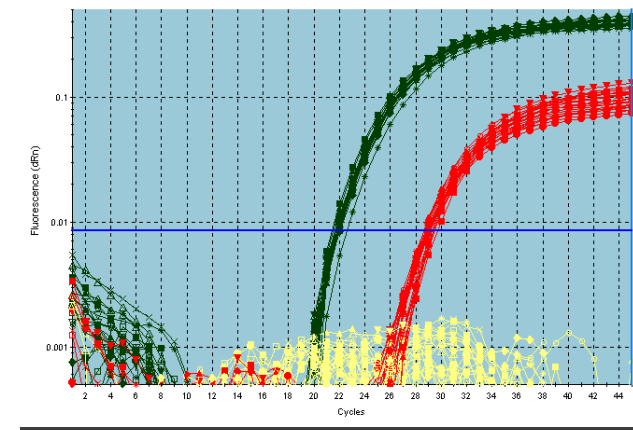
- HCV plasma samples were value-assigned with quantitative VERSANT[®] HCV RNA assay (bDNA)

Panel Number	Concentration (copies/mL)
LP1	10,000,000
LP2	1,000,000
LP3	100,000
LP4	10,000
LP5	1,000
LP6	100
LP7	50
LP8	25
Negative Serum	0
Negative Plasma	0

Specificity and Imprecision

■ 100% Specificity

No false positives could be detected when copurified with neighbouring high positive samples



■ Good Reproducibility

- ◆ within run
- ◆ between run

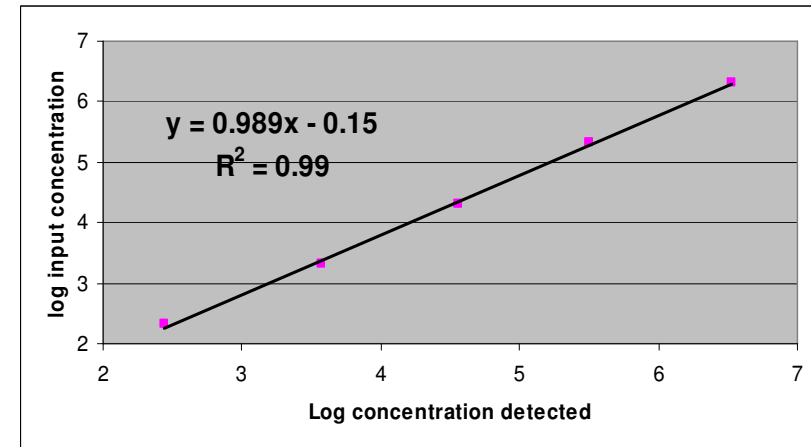
CV's < 25% over most of the dynamic range

Linearity and Sensitivity

■ Linearity

at least 5 magnitudes

from 10^7 down to 10^2 HCV copies/ml



■ Recovery / Purification Efficiency

80 - 100 %

■ Sensitivity / LOD (95% hit rate)

with 500 μ l Plasma

55 HCV copies / ml
(11 IU / ml)

Universal Applicability

Nucleic Acid Purification protocols designed for

■ Pharmacogenomic DNA Testing

- ◆ Cystic Fibrosis Testing
- ◆ Predictive SNP's for Cardiovascular Risk Assessment

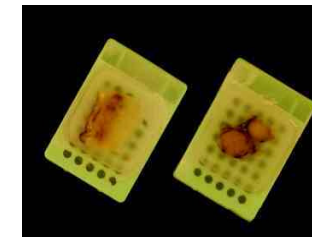
Sample: EDTA Blood



■ Oncology Testing

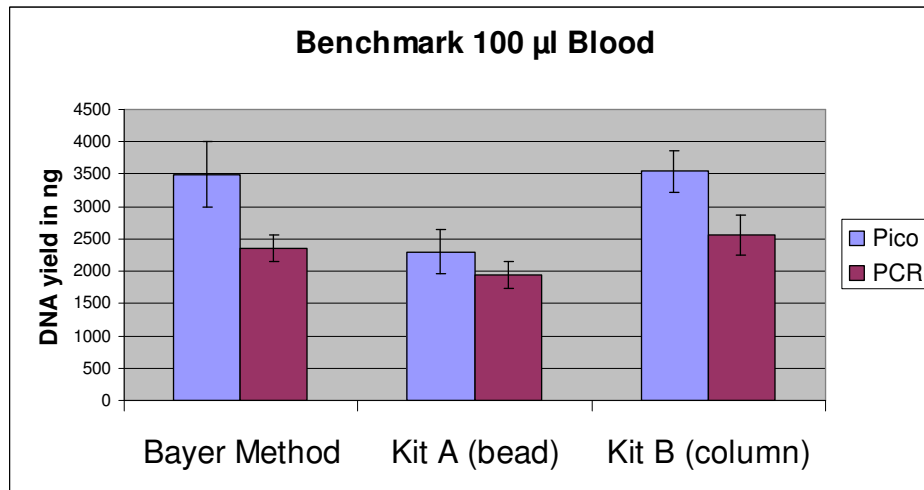
- ◆ Predictive RNA Marker Panels
for Individualized Therapy

Sample: Fixed Paraffin-Embedded (FPE) Tissue

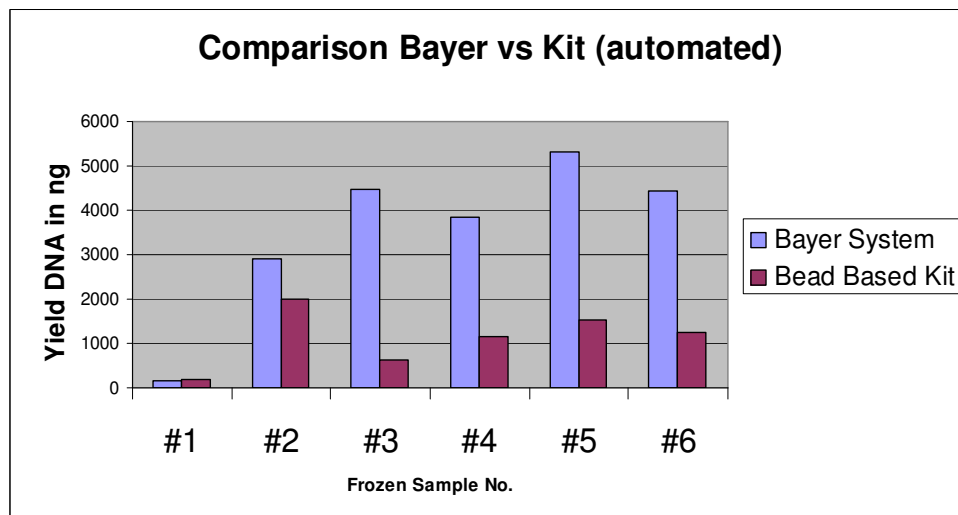


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Benchmark DNA Isolation from Blood

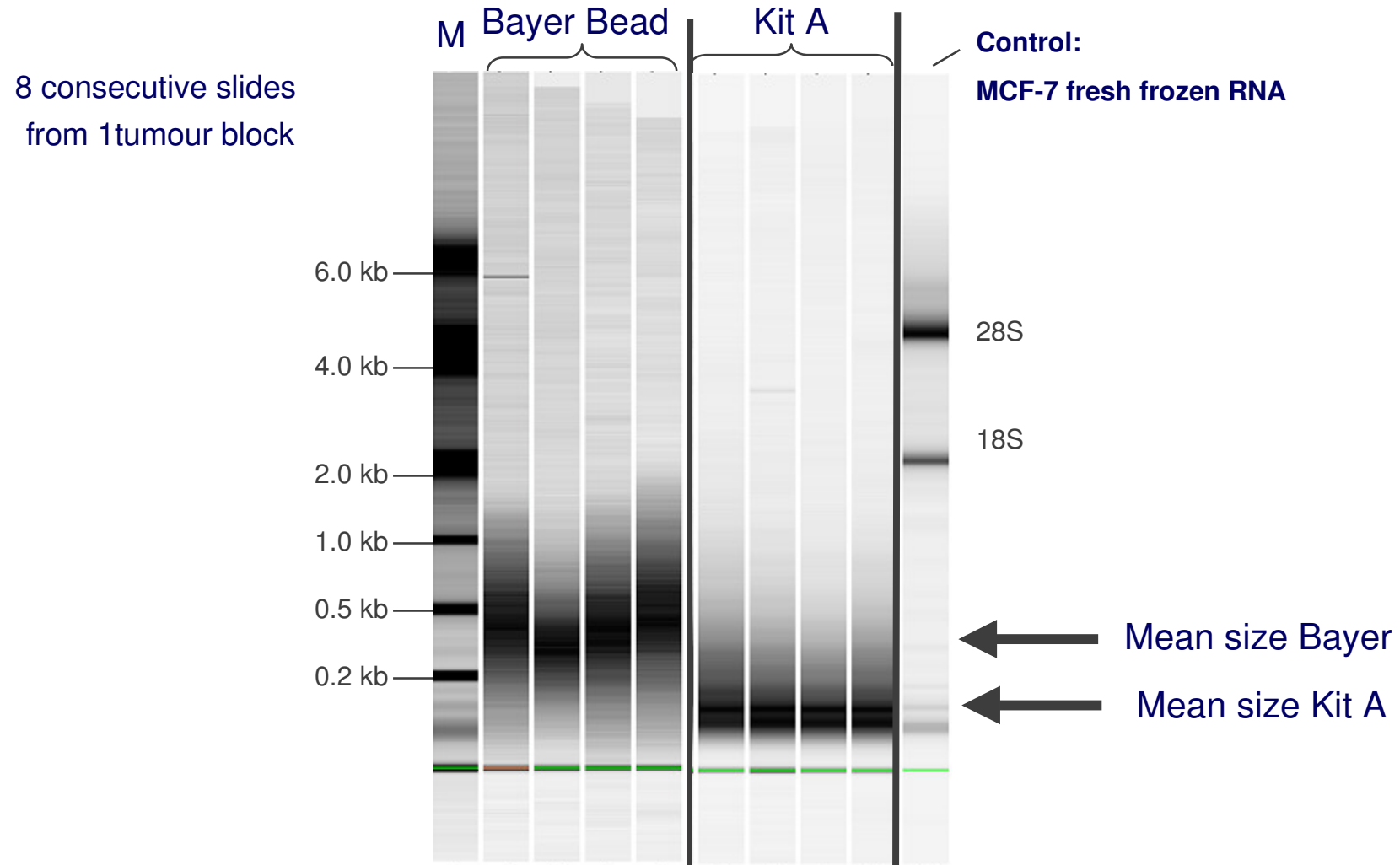


- Manual Isolation from 100 µl EDTA blood showed comparable yields for Bayer and two commercial kits



- Automated Side to Side Benchmark on standard liquid handling platform showed much better yields with Bayer method compared to commercial bead kit

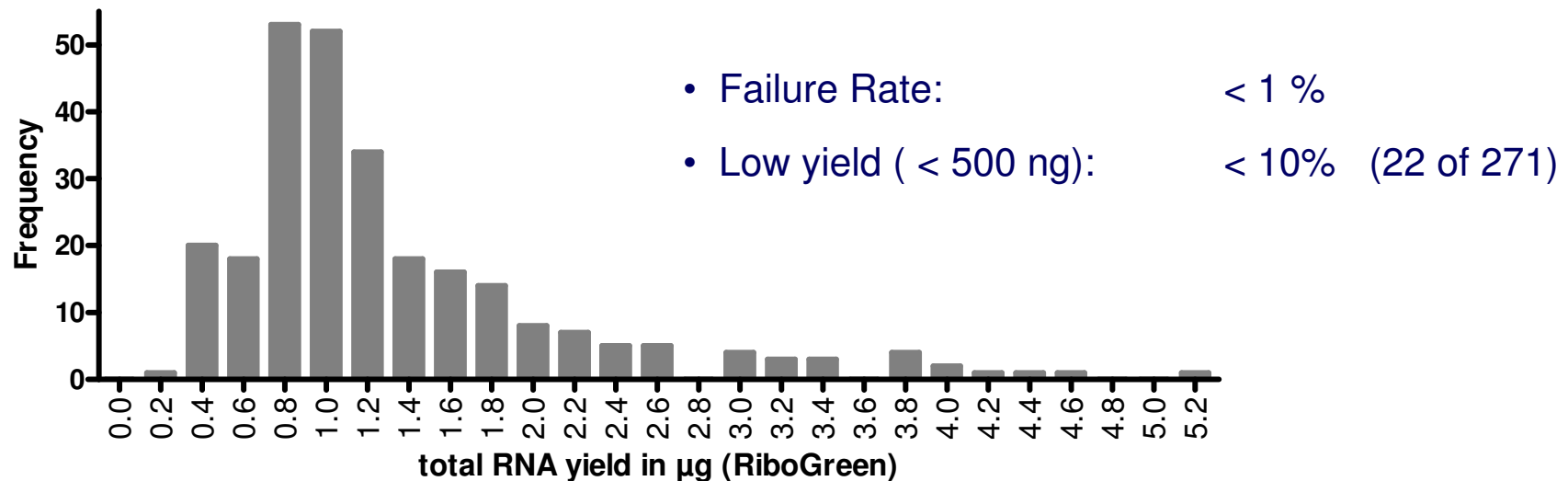
RNA Fragment Size Bayer vs. Column Kit



Archived FPE tissue RNA is fragmented (100-500 bp)

Yield of Total RNA from Archived FPE Slides

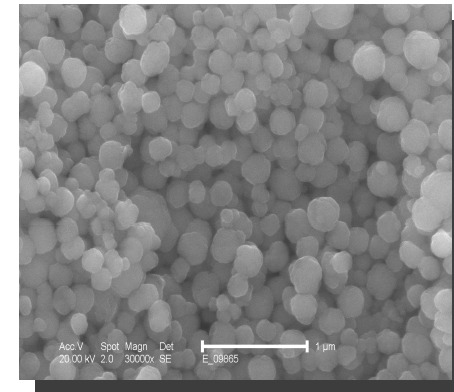
RNA from about 271 clinical breast cancer samples (10 μ m slides up to 8 years old) was isolated with manual Bayer protocol



Expression profiling of predictive/prognostic marker panels with qRT-PCR on archived FPE tissue RNA feasible

Summary

- Robust and new Bayer magnetic particles
- Essential part of Bayer's detection platforms
- Sensitive detection assays for HCV, HIV, HBV and CT/GC
- Efficient and quantitative procedure for automated isolation of HCV + HIV
- Flexible in its applications for
 - ◆ Infectious disease testing
 - ◆ Pharmacogenomics
 - ◆ Tumour diagnostics





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