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

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Diagnostics

Bayer HealthCare Divisions



Diagnostics Research, Leverkusen

Pharma

Diagnostics

Diabetes Care

Consumer Care

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

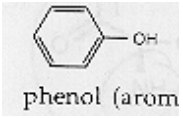
  - Pure nucleic acids / Interference free
  - Process automation
  - Sensitive with high recovery/purification efficiency

Clinical Need for Technical Performance

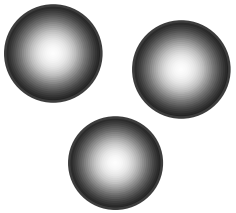
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History of Nucleic Acid Isolation

- Phenol / Chloroform
  - Special case
- Column Technology (Silica particles)
  - Research (automation difficult)
- Silica-coated magnetic particles
  - In Vitro Diagnostic







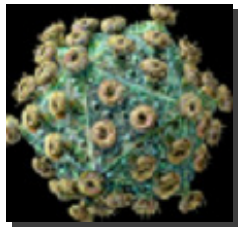
Innovation

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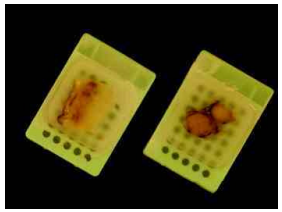
Advantages of Magnetic Particle Technology

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





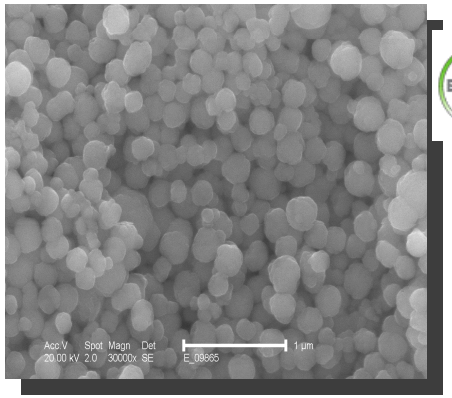




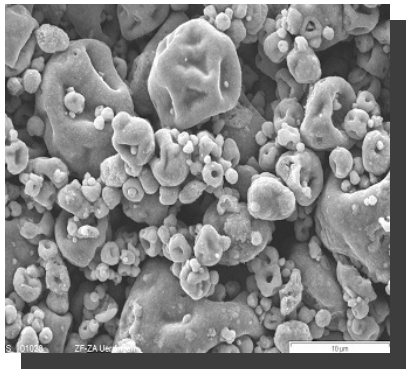
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Bayers „new“ Magnetic Particles

- „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



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Others

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Properties Determine Performance

Properties	Automation Advantage
■ „homogenous“ particle size distribution	Reproducibility
■ small particles (< 1 µm)	optimal suspension behaviour
■ very high iron content	efficient magnetization
■ very good paramagnetism	efficient isolation
■ high storage stability	Reliability

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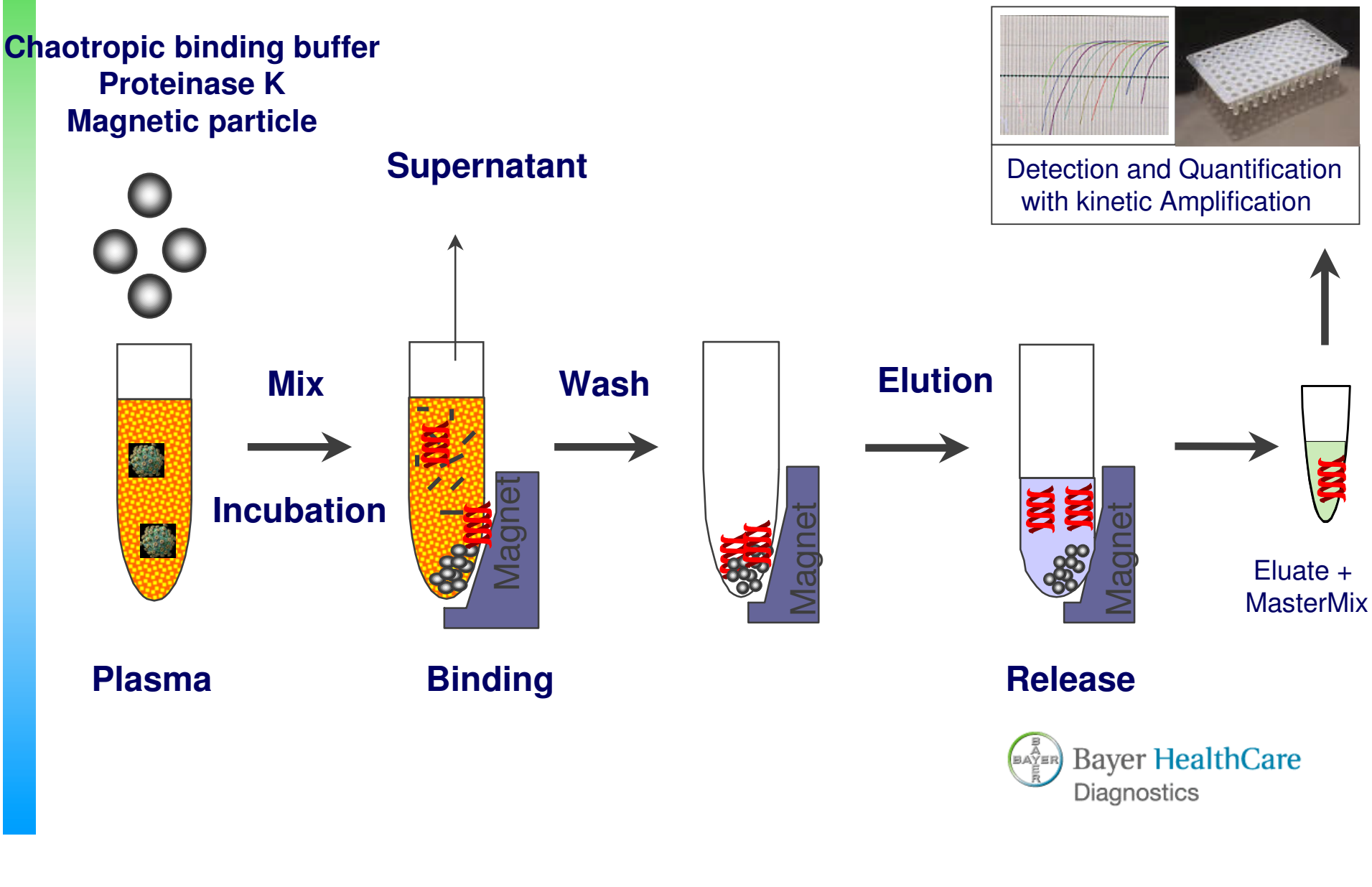
Infectious Disease Testing Assay Portfolio

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

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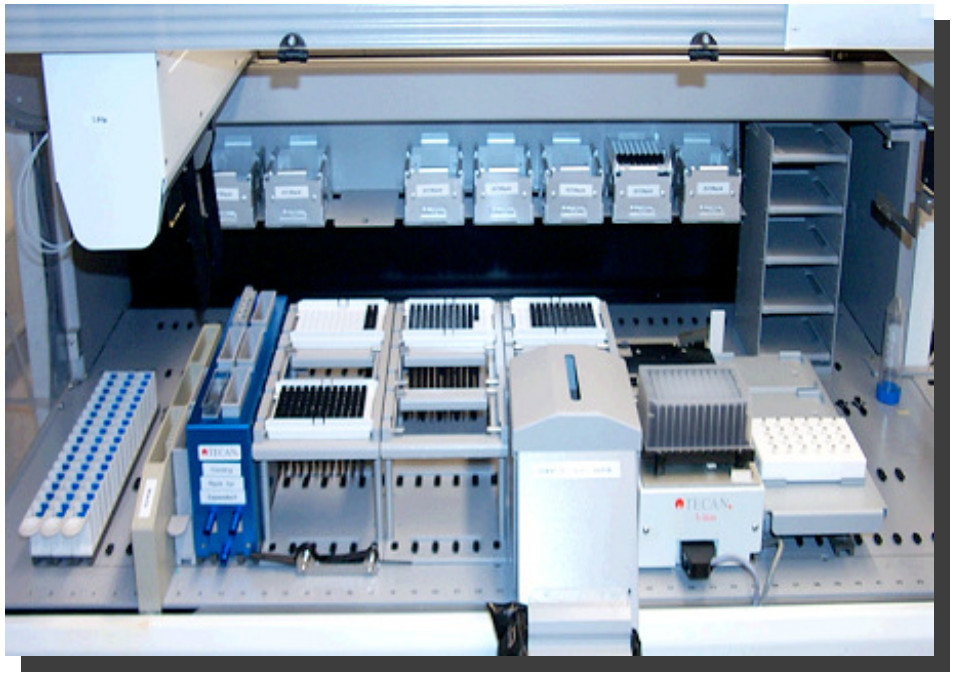
Schematic Nucleic Acid Isolation Protocol



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Automation of HCV Isolation (Model System)

- Commercial Liquid Handling Robot
- Module
  - Samples
  - Reagents
  - Tips
  - Waste
  - Microtiter plate
  - Shaker
  - Heating block
  - Magnet
- Complete automation



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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 µl or 1 ml

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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

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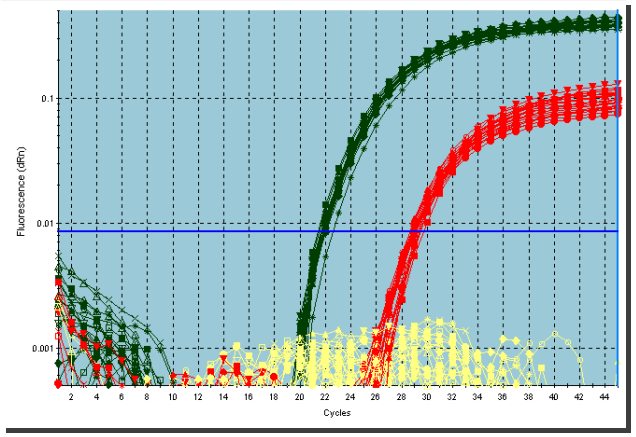
Specificity and Imprecision

- 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

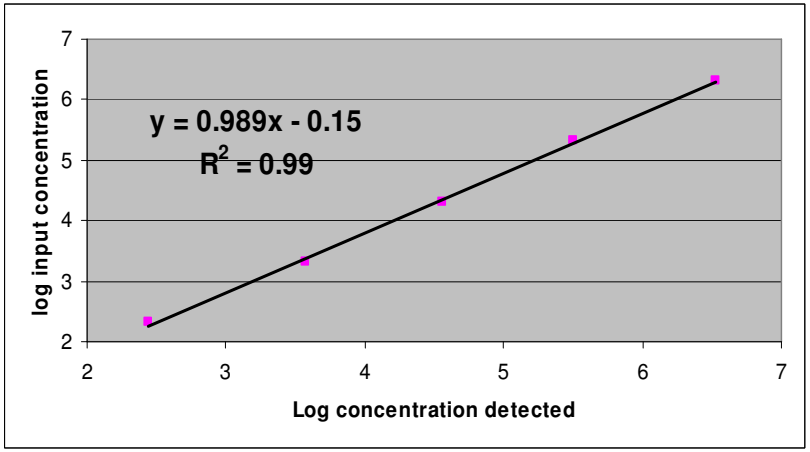
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Linearity and Sensitivity

- 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 µl Plasma

  55 HCV copies / ml
  (11 IU / ml)

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Universal Applicability

Nucleic Acid Purification protocols designed for

- Pharmacogenomic DNA Testing
  - Cystic Fibrosis Testing
  - Predictive SNP´s for Cardiovascular Risk Assessment

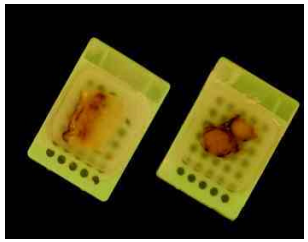
  Sample: EDTA Blood



- Oncology Testing
  - Predictive RNA Marker Panels

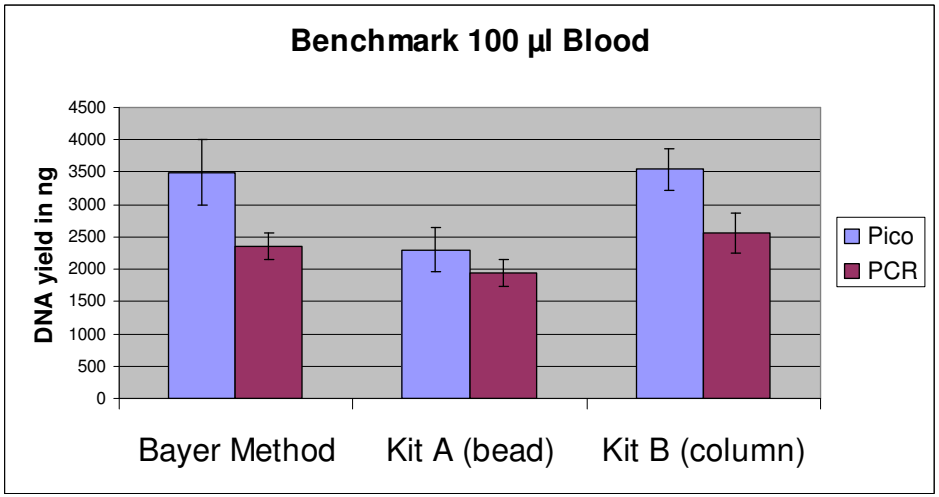
  for Individualized Therapy

  Sample: Fixed Paraffin-Embedded (FPE) Tissue

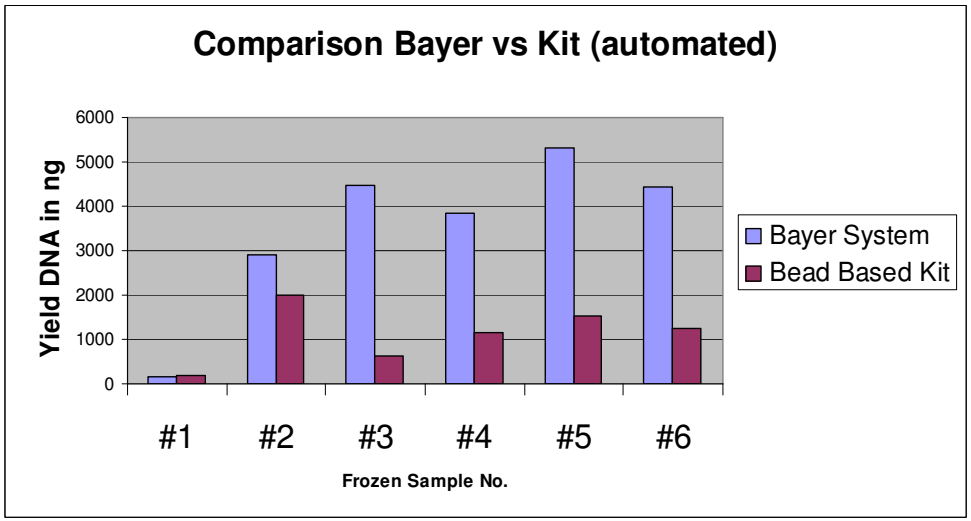


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Benchmark DNA Isolation from Blood



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

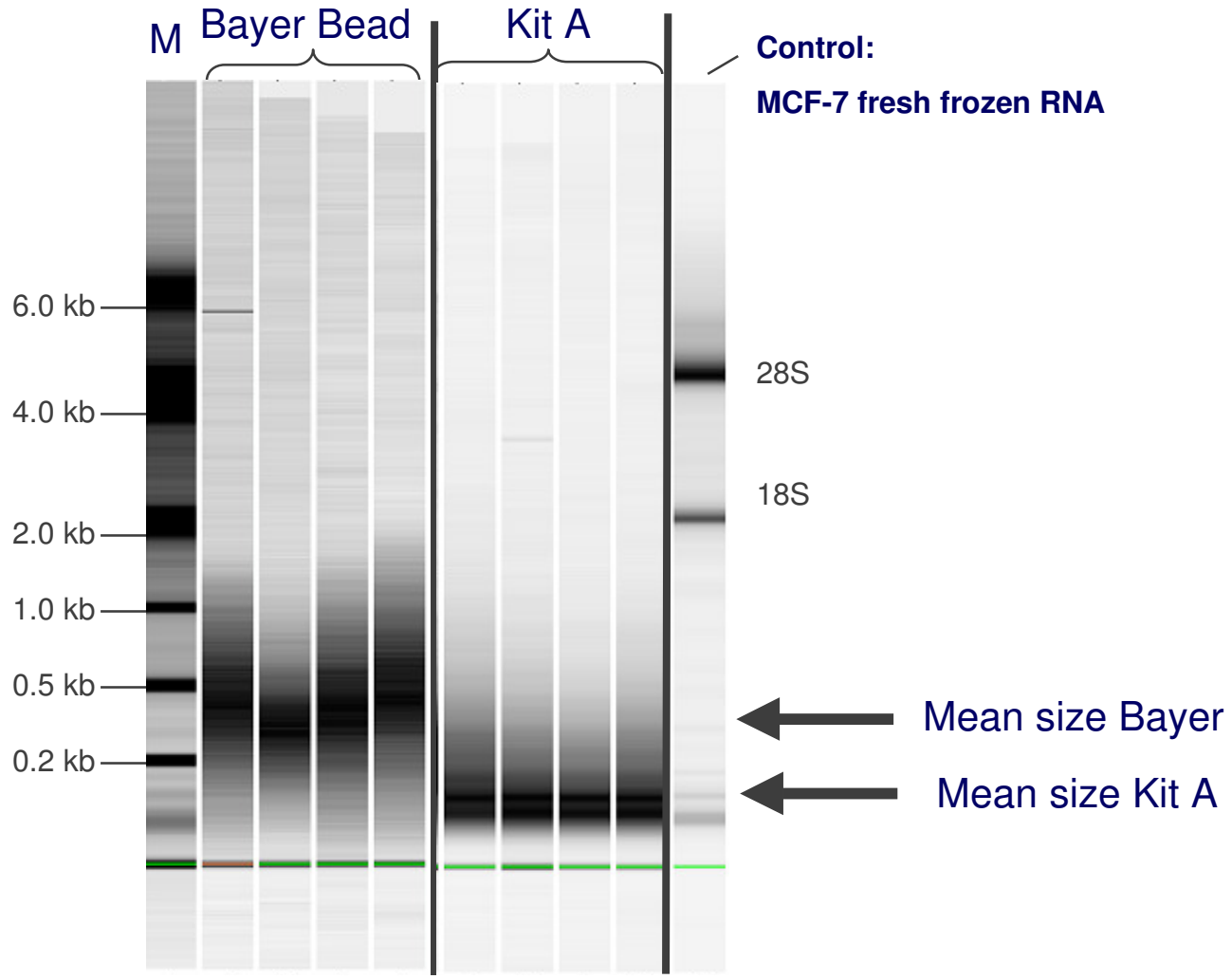


- Automated Side to Side Benchmark on standard liquid handling platform showed much better yields with Bayer method compared to commercial bead kit

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RNA Fragment Size Bayer vs. Column Kit

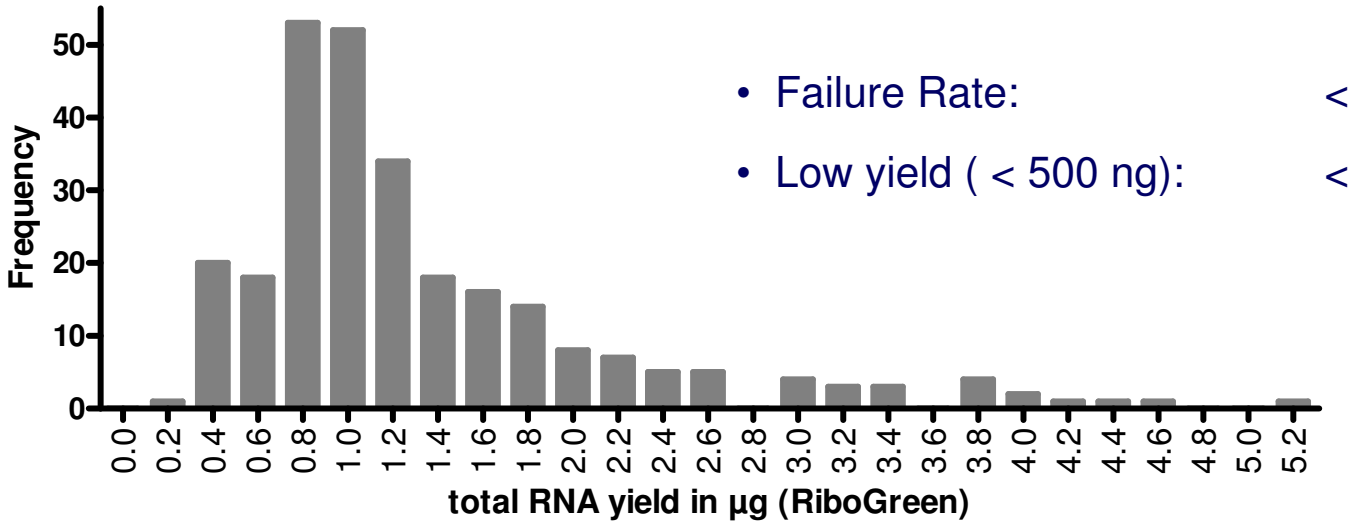
8 consecutive slides
from 1tumour block



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 µm slides up to 8 years old) was isolated with manual Bayer protocol



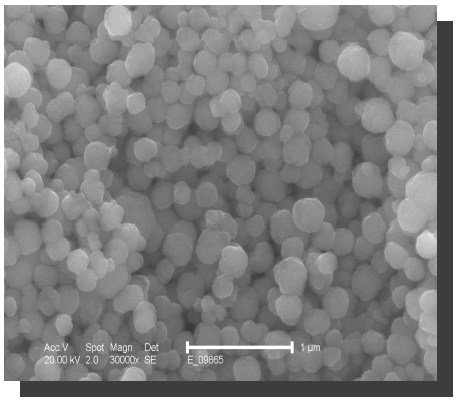
- Failure Rate: < 1 %
- Low yield (< 500 ng): < 10% (22 of 271)

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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