

Lec 5

Medical tests and digital assays

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Main content:

1. Introduction to in vitro diagnostics
2. Medical Laboratory Methodology
3. Digital Immunoassay
4. Digital nucleic acid test

01

What is in vitro diagnosis



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In Vitro Diagnostics: A Crucial Component of Medical Practice



Hospital Visit



Patient Registration

Diagnosis

Prescribing Treatment Plans



In Vitro Diagnostics



Medical Imaging



Ultrasound

Medical tests

**The starting point
for medical services**



**An essential tool for the development of
evidence-based medicine**

Determine the patient's disease
profile and severity

Changes in diagnostic indicators were used
to evaluate the efficacy of the drug
Create opportunities for follow-up
medical services

The diagnosis is the doctor's eye, and in
vitro diagnostics can provide 70-80% of the
diagnostic information for the clinic

Run a
fever



Symptoms

Body temperatures vary slightly from person to person and at different times of day. The average temperature has traditionally been defined as 98.6 F (37 C). A temperature taken using a mouth thermometer (oral temperature) that's 100 F (37.8 C) or higher is generally considered to be a fever.

In vitro diagnostic classification

发烧



体外诊断分类

Adults

Call your health care provider if your temperature is 103 F (39.4 C) or higher. Seek immediate medical attention if any of these signs or symptoms accompanies a fever:

- Severe headache
- Rash
- Unusual sensitivity to bright light
- Stiff neck and pain when you bend your head forward
- Mental confusion, strange behavior or altered speech
- Persistent vomiting
- Difficulty breathing or chest pain
- Abdominal pain
- Pain when urinating
- Convulsions or seizures

发烧

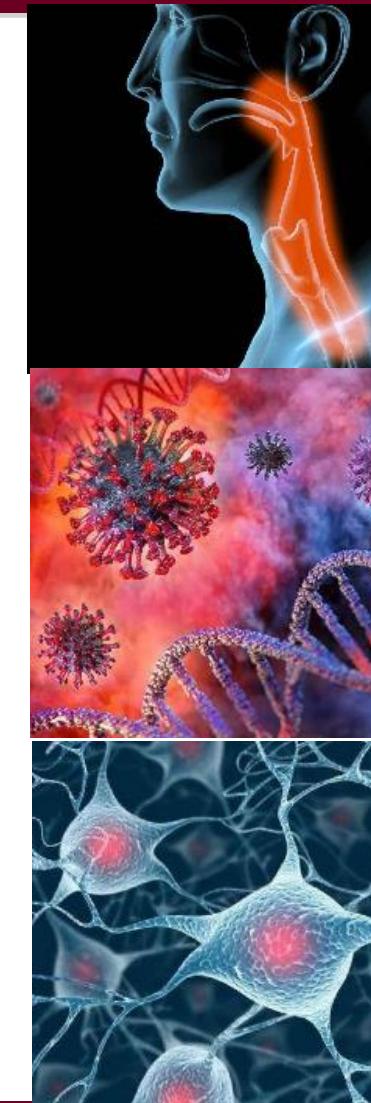
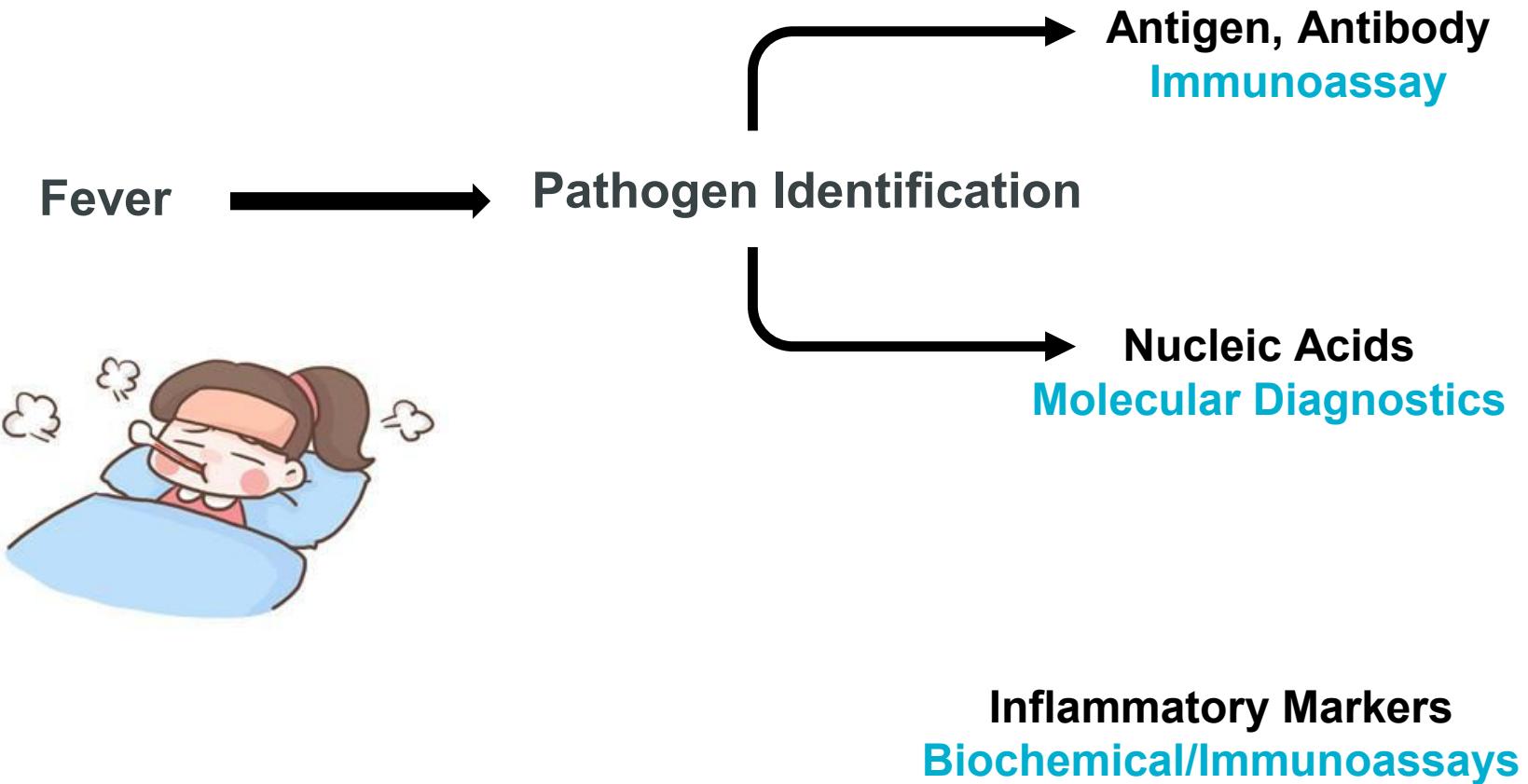


体外诊断分类

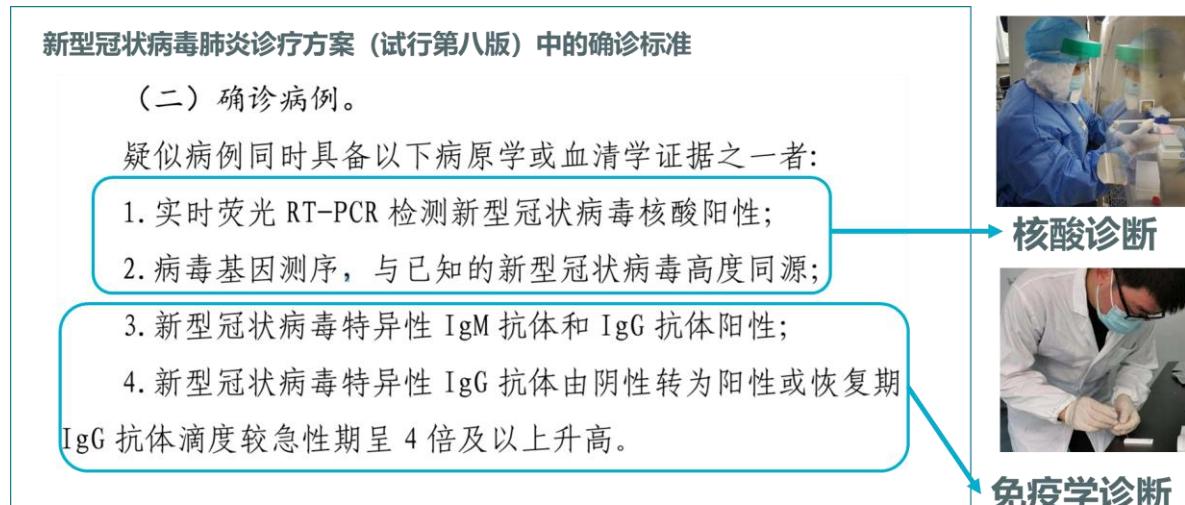
Fever or elevated body temperature might be caused by:

- A viral infection
- A bacterial infection
- Heat exhaustion
- Certain inflammatory conditions such as rheumatoid arthritis — inflammation of the lining of your joints (synovium)
- A cancerous (malignant) tumor
- Some medications, such as antibiotics and drugs used to treat high blood pressure or seizures
- Some immunizations, such as the diphtheria, tetanus and acellular pertussis (DTaP), pneumococcal or COVID vaccine

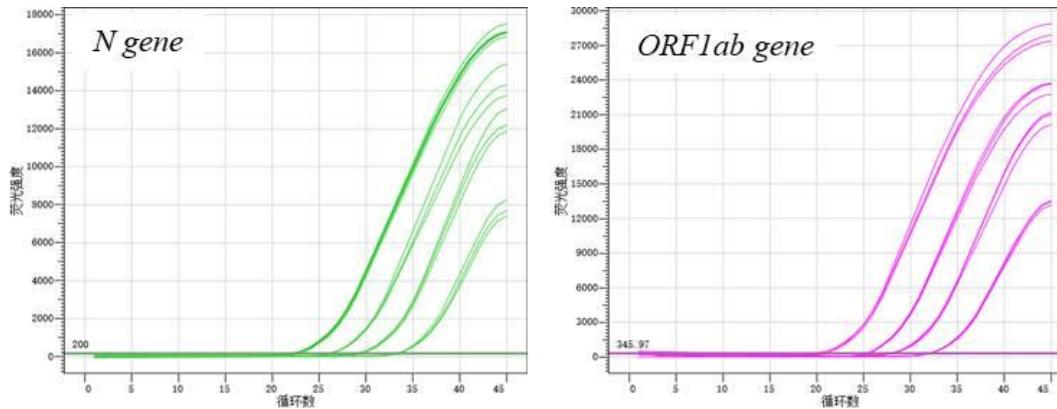
Molecular Diagnostics and Immunoassays



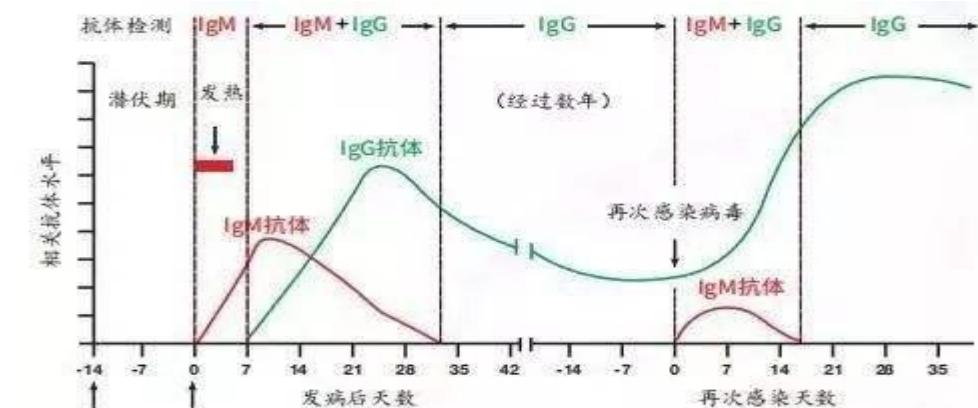
IVD is used in COVID testing



诊疗方案中四种确诊方法均为体外诊断方法



The base sequence of the virus is detected for diagnosis

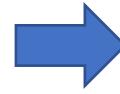


Diagnosis is made by measuring the level of antibodies produced by the body's immune system

What is IVD

Definition of in vitro diagnostics

In Vitro Diagnostics (IVD): Tests performed outside the body on samples like blood, fluids, or tissues to provide clinical information for disease diagnosis or functional assessment.



取样本

IVD检测

结果分析&治疗决策

In Vivo Diagnostics: Tests performed inside the body using physical or electronic methods (e.g., X-ray, CT, MRI, ultrasound) to obtain diagnostic and monitoring information.



3 • 代表性的医疗仪器

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↓ Clinical Biochemistry Tests

Tests Performed by Automated Clinical Chemistry Analyzer

Liver Function:

- Alanine aminotransferase (**ALT**, also known as GPT)
- Aspartate aminotransferase (**AST**, also known as GOT)
- Alkaline phosphatase (**ALP**)
- Total bilirubin (**TBIL**)
- Direct bilirubin (**DBIL**)
- Total protein (**TP**)
- Albumin (**ALB**)

Renal (Kidney) Function:

- Blood urea nitrogen (**BUN**)
- Creatinine (**Cr** or **CREA**)
- Carbon dioxide combining power (**CO₂**)
- Uric acid (**UA**)

全自动生化分析仪



血气分析仪



POCT型血糖仪

Lipids:

- Total cholesterol (**TC** or **CHOL**)
- Triglycerides (**TG**)
- High-density lipoprotein cholesterol (**HDL-C**)
- Low-density lipoprotein cholesterol (**LDL-C**)

Glucose:

- Glucose (**GLU**)

Cardiac Markers:

- Creatine kinase (**CK**)
- Creatine kinase-MB isoenzyme (**CK-MB**)
- Lactate dehydrogenase (**LDH**)
- α -Hydroxybutyrate dehydrogenase (α -**HBDH**)
- γ -Glutamyl transferase (**GGT**, sometimes written as γ -**GT**)

Electrolytes / Ions:

- Calcium (**Ca**)
- Phosphorus (**P**, often reported as inorganic phosphate, **Pi**)
- Magnesium (**Mg**)
- Potassium (**K**)
- Sodium (**Na**)
- Chloride (**Cl**)

全自动生化分析仪



POCT型血糖仪



Blood tests



Urine test



Are these in vitro diagnostic techniques (IVD)?



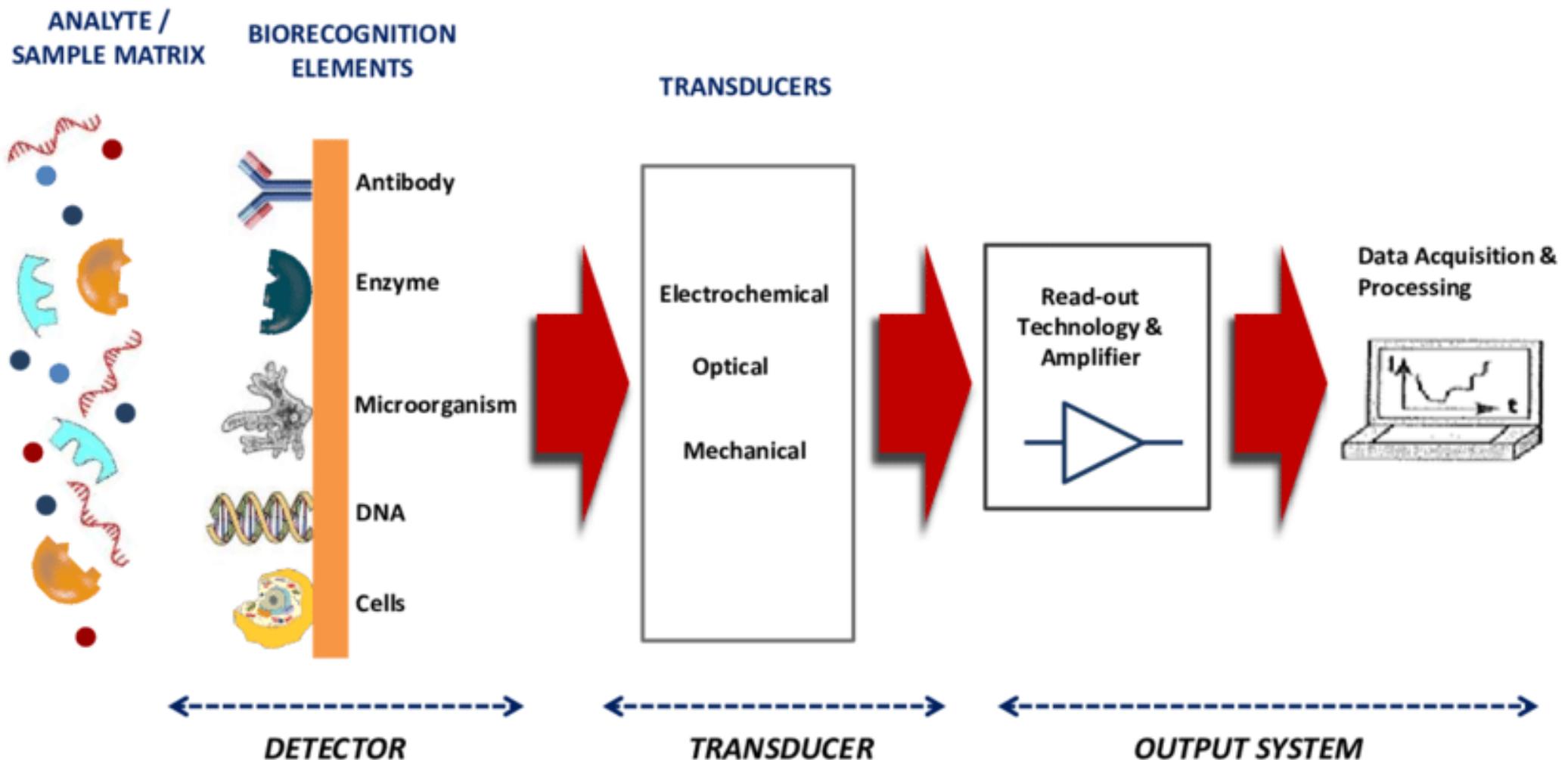
Nucleic acid testing



Secretion testing



Detection principle (biosensing)



In vitro diagnostic
techniques



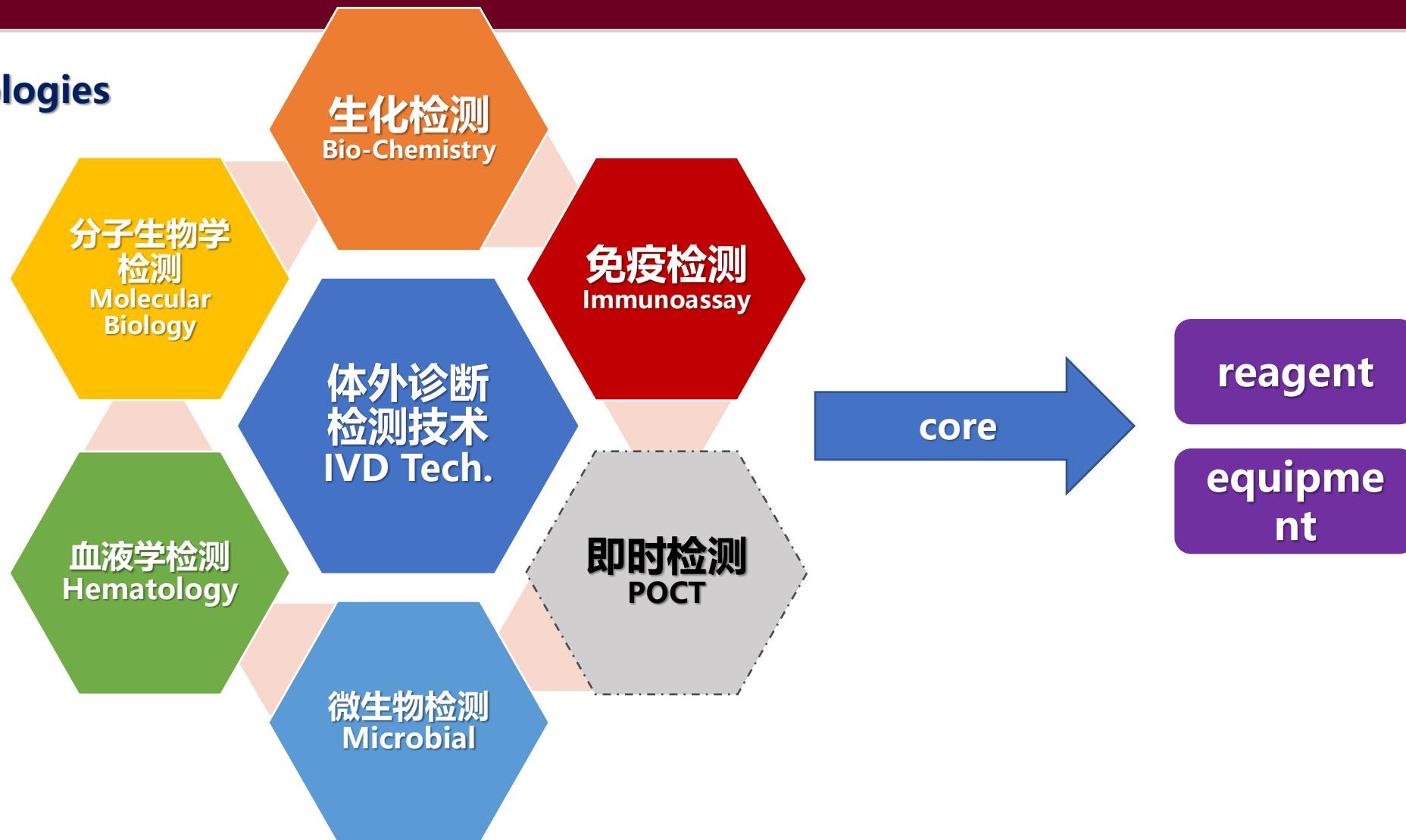
体外诊断系统



In vitro diagnostic tests

Step into IVD technology

Classification of IVD technologies





Biochemistry a



Chemiluminescence
immunoassay analyzer



Blood cell analyze



Urine sediment
analyzer



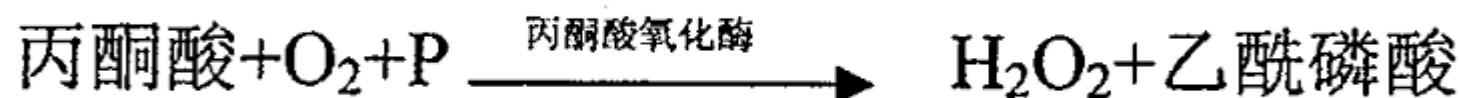
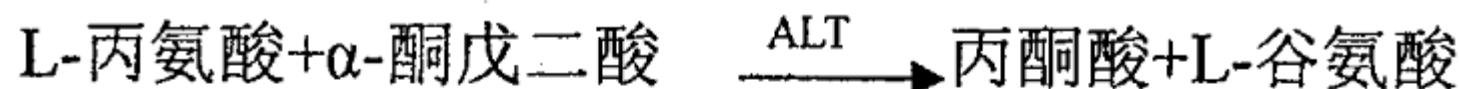
Blood gas
analyzer



Fully automated
in-house culture
analyzer

The inspection items of the automatic biochemical instrument

- **Liver function:** 谷丙转氨酶 (ALT/GPT) 、谷草转氨酶(AST/GOT) 、碱性磷酸酶 (ALP) 、总胆红素 (T.BIL) 直接胆红素 (D.BIL) 、总蛋白 (TP) 、白蛋白 (ALB)
- **Kidney function:** 尿素氮 (BUN) 、肌酐 (Cre) 、二氧化碳结合力 (CO2) 、尿酸 (UA)
- **blood lipids:** 总胆固醇 (CHO) 、甘油三脂 (TG) 、高密度脂蛋白胆固醇 (HDL-C) 、低密度脂蛋白胆固醇 (LDL-C)



uric acid



Uric acid test

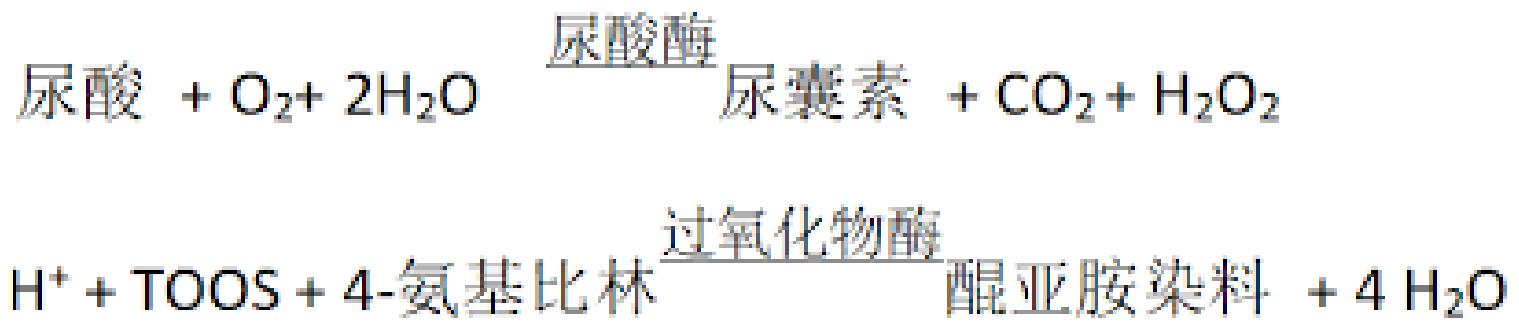


尿酸(UA)检测试剂盒
BB-474342

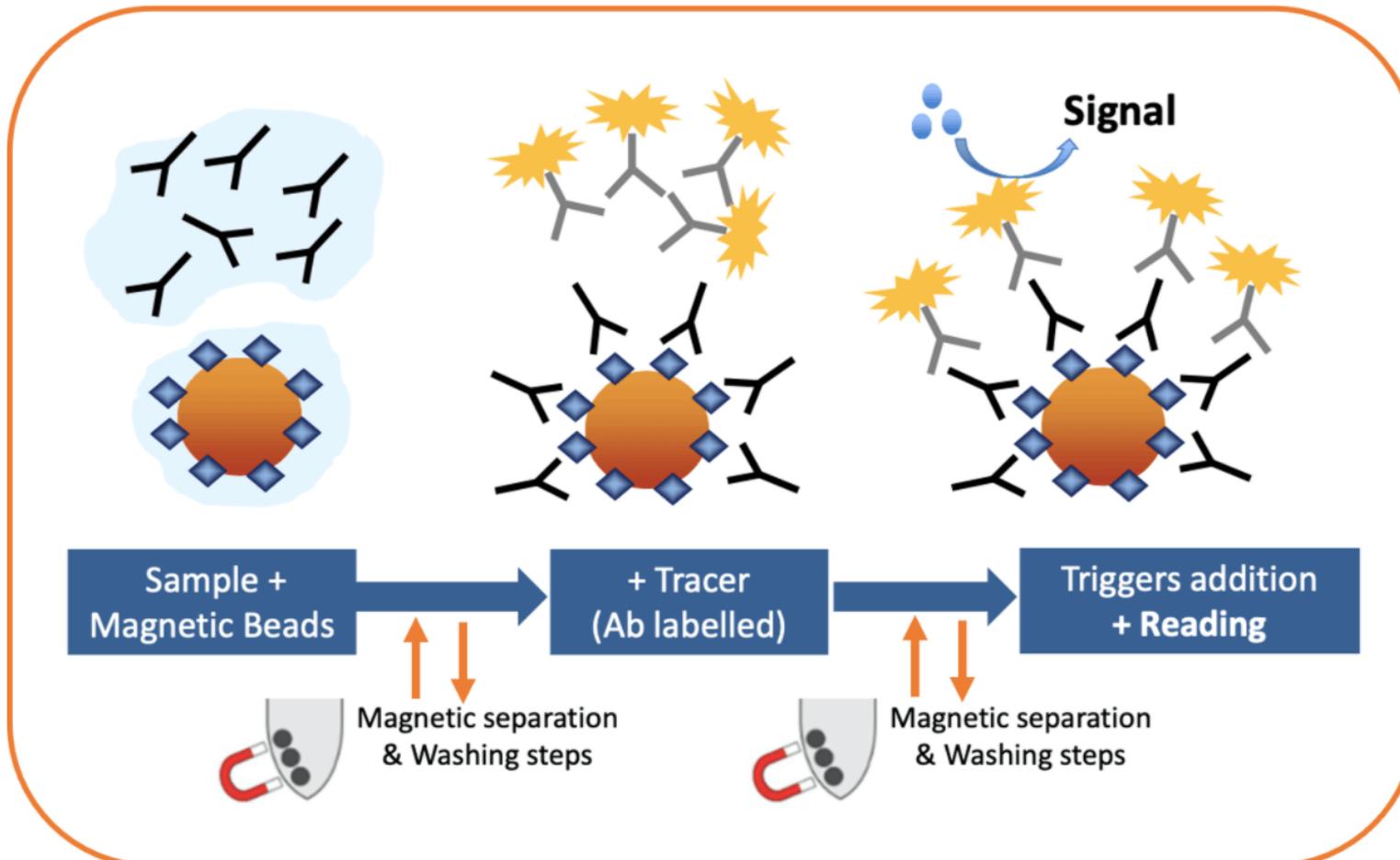
选择规格 100T

价格 ￥520元

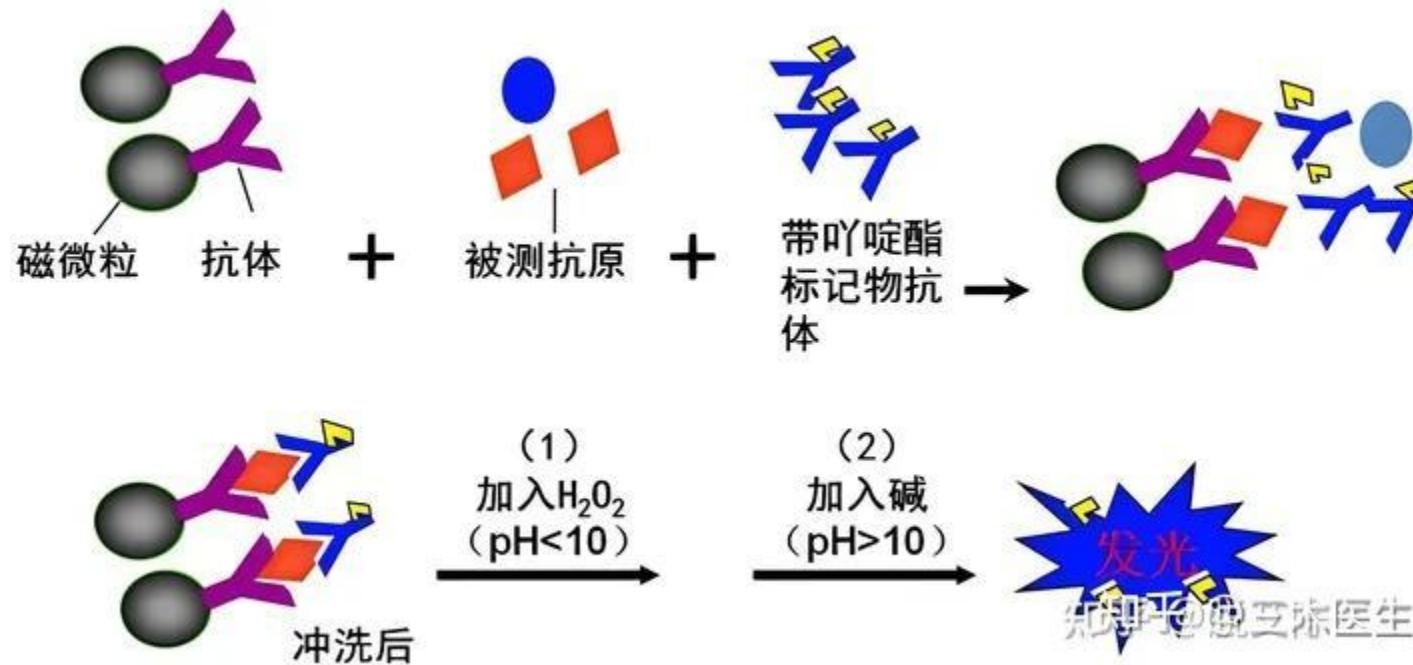
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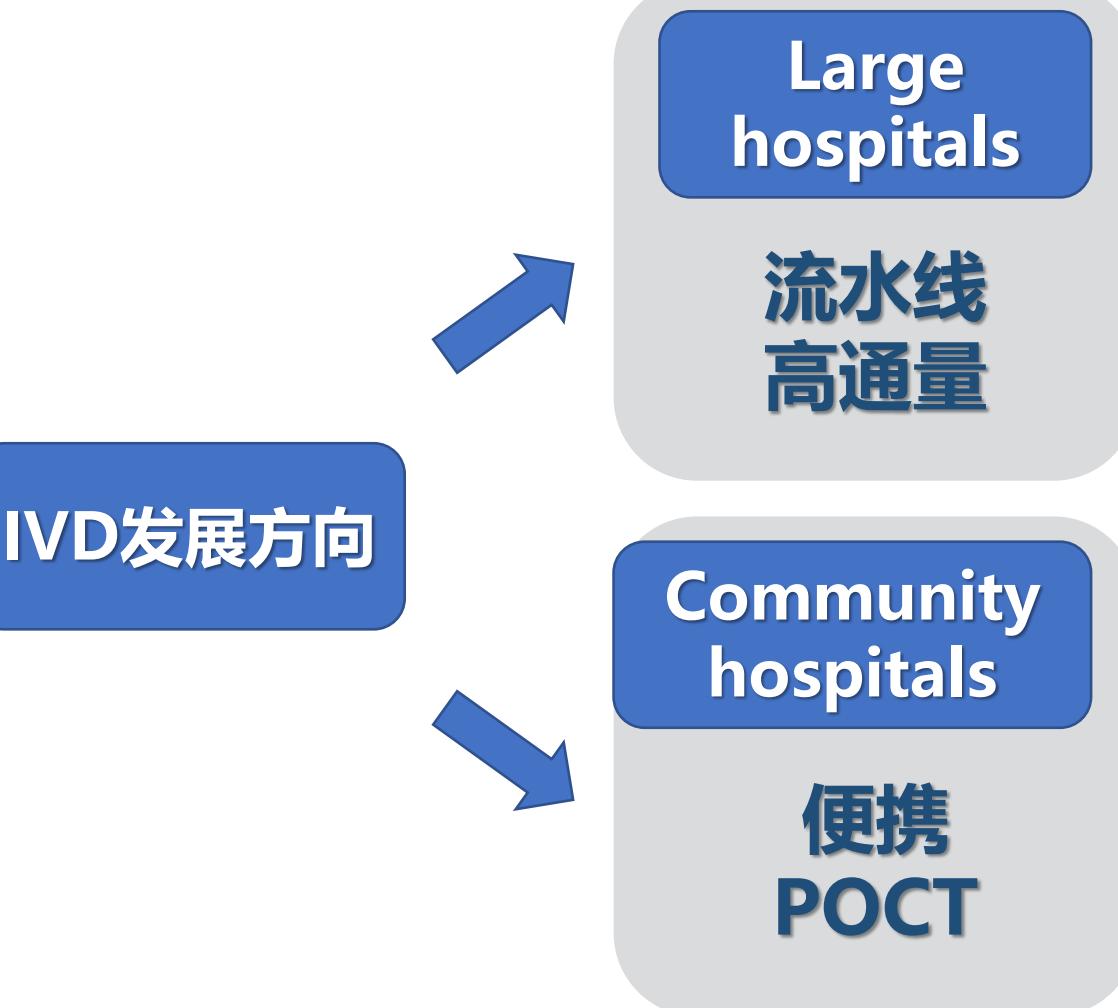
Chemiluminescence immunoassay analyzer



For example, HIV testing



IVD Two major trends



全自动化学发光免疫分析仪
MAGLUMI X8

高速：速度600T/H
高通量：样本位300个
高可靠性：管式直接化学发光
高智能化：可互联自动化系统



活水快易准
PTL40全能检验工作站

单人份试剂
同时检测多个项目同时出结果
生化、胶体金免疫、荧光免疫、
化学发光免疫检测集于一身

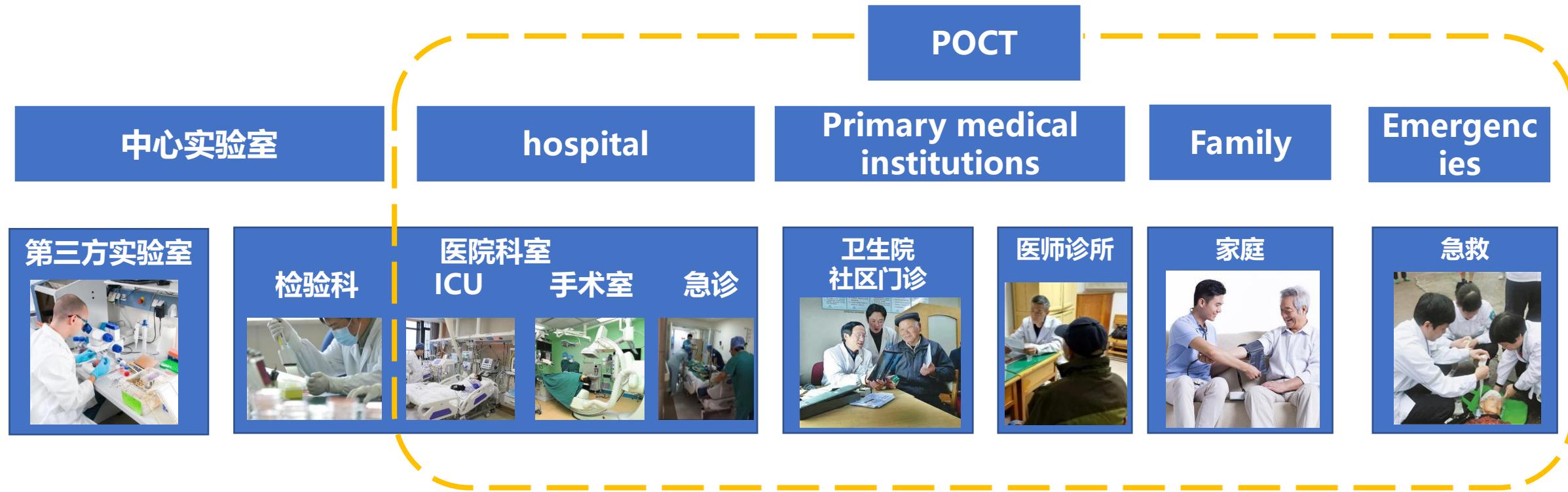
【罗氏-Explore personalised automation of the molecular laboratory 分子诊断流水线】

https://www.bilibili.com/video/BV1jT4y1A78z?share_source=copy_web&vd_source=83cb43b5b39d7eec01e40aabbef93ae4

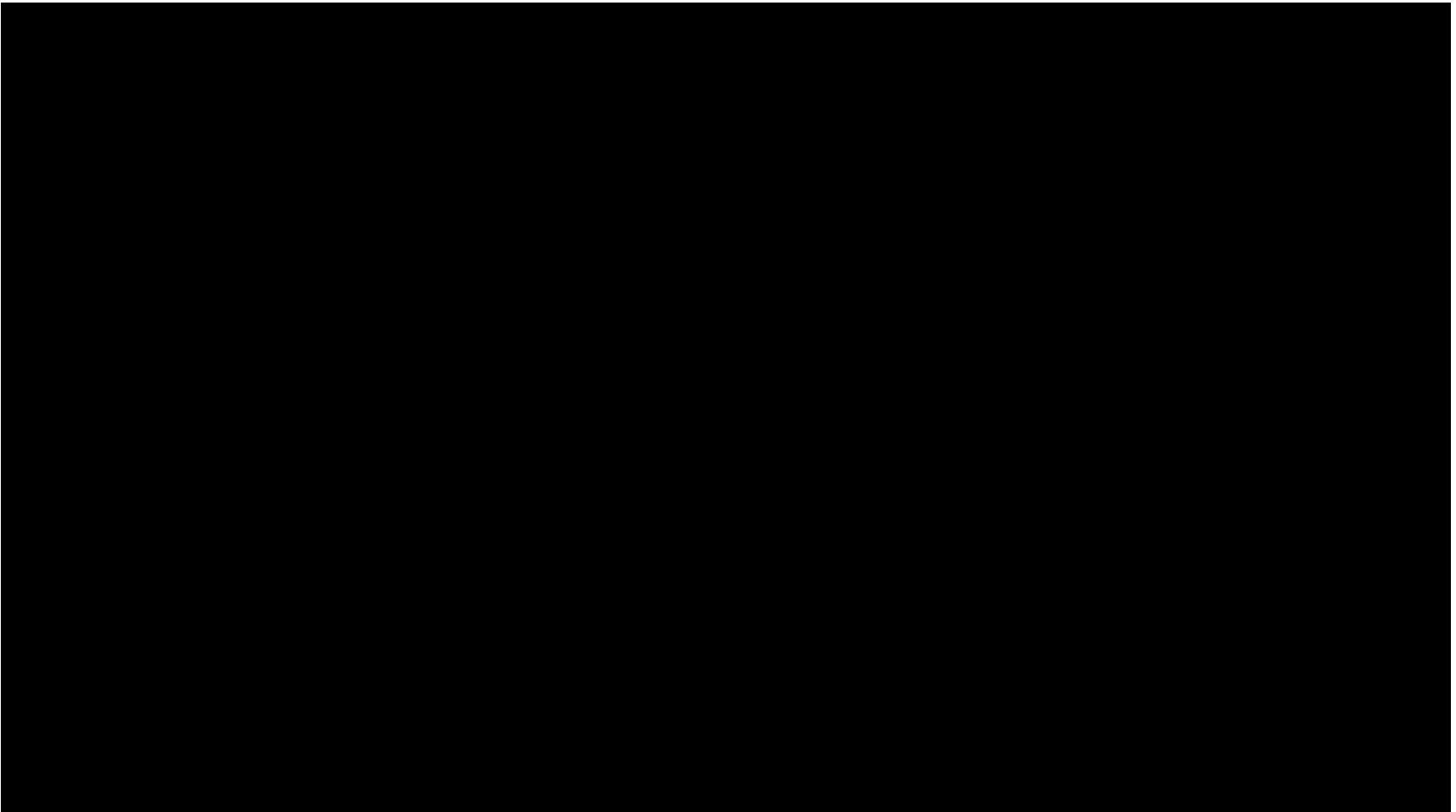
IVD两大发展趋势——即时诊断

POCT即时诊断是指在病人旁边进行的快速诊断，是在采样现场即刻进行分析，省去了标本在实验室检验时的复杂处理程序，是快速得到检验结果的一类新方法。

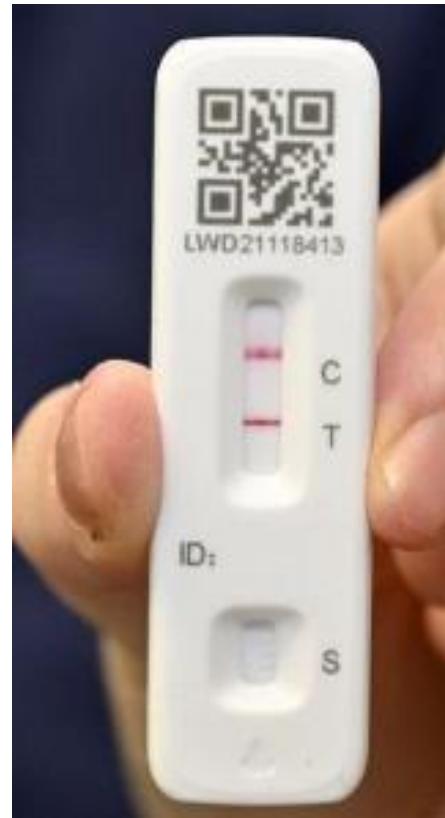
与专业实验室诊断相比，POCT仅保留了最核心的“样本收集—样本分析—质量控制—得出有效结果—解释报告”步骤，从而缩短了诊断时间，适合于在医院ICU、手术、急诊、诊所及患者家中使用。



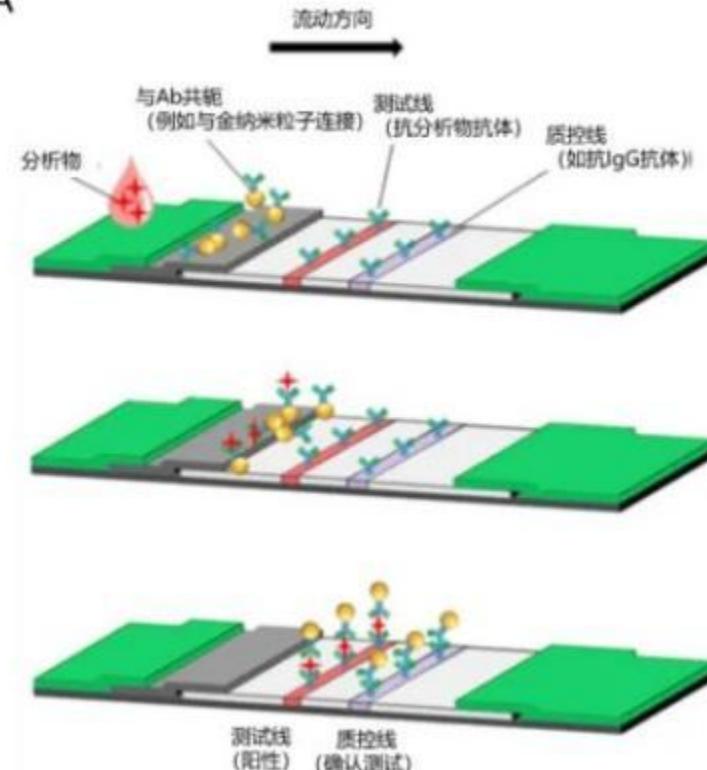
Examples of POCTs



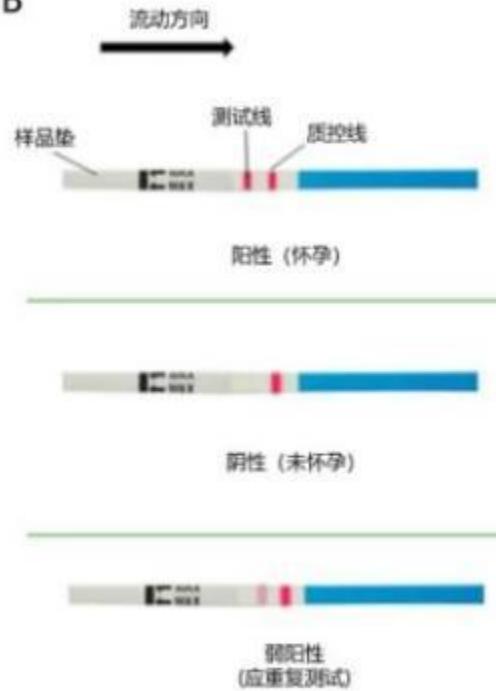
POCT 的例子



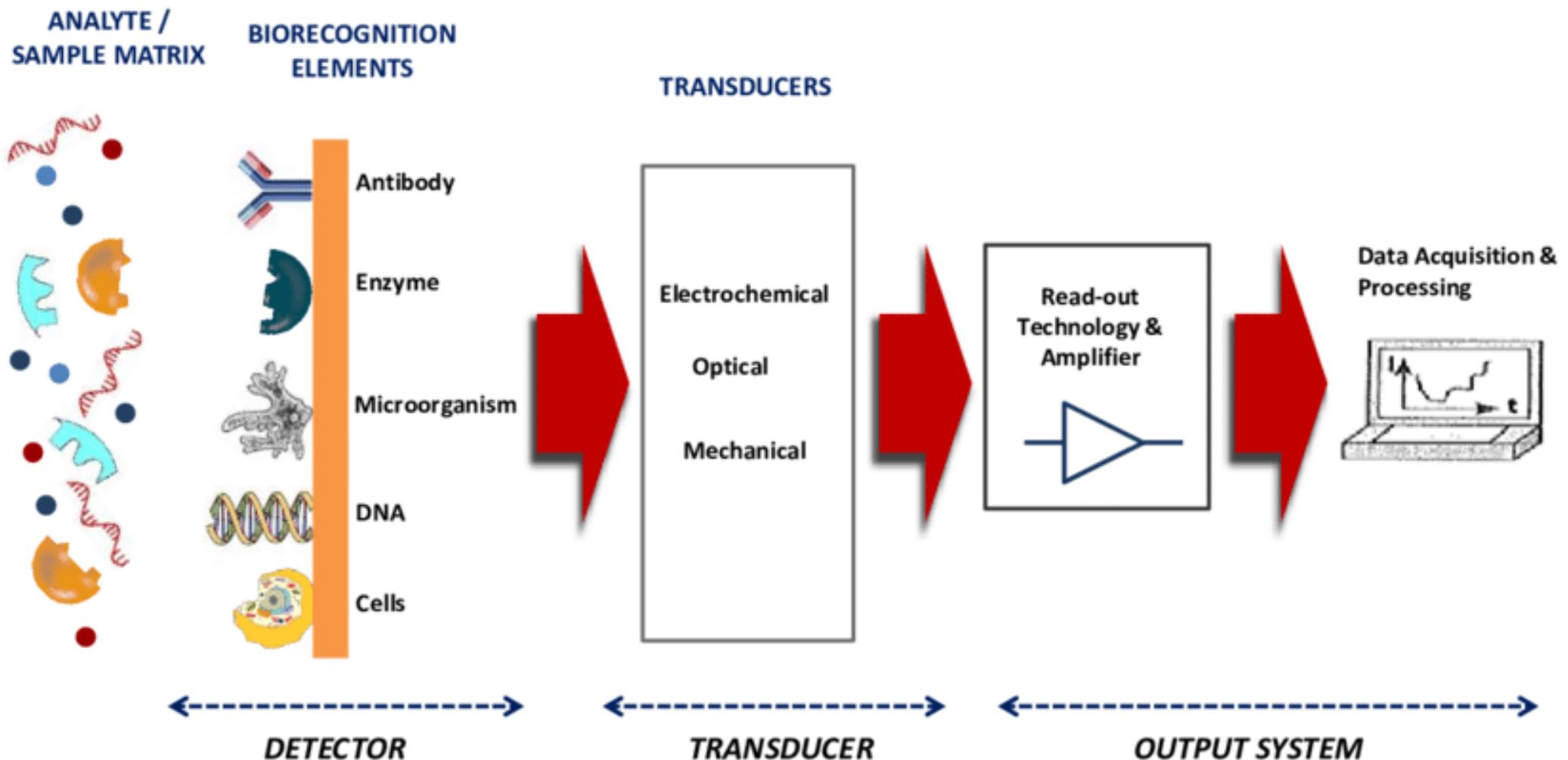
A



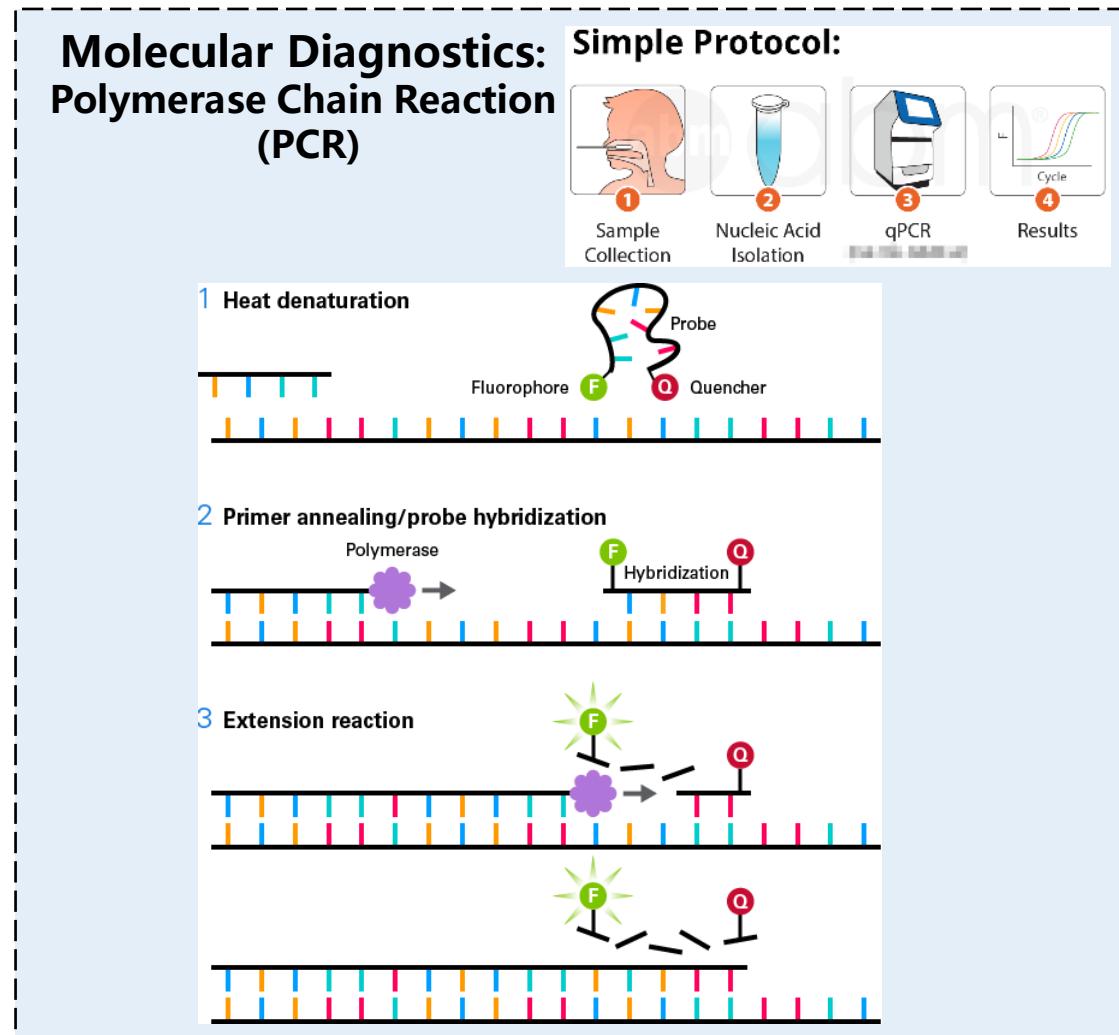
B



Detection strategy

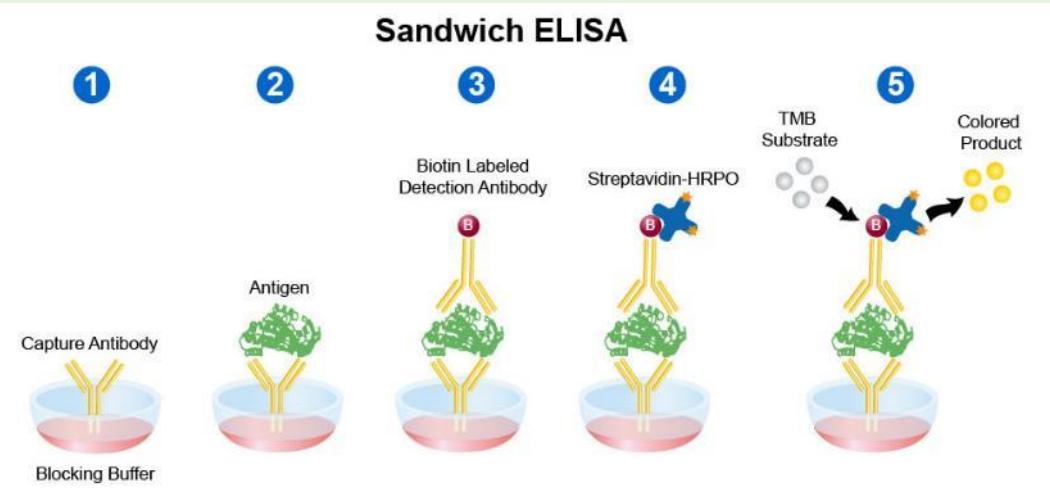


Detection Principles

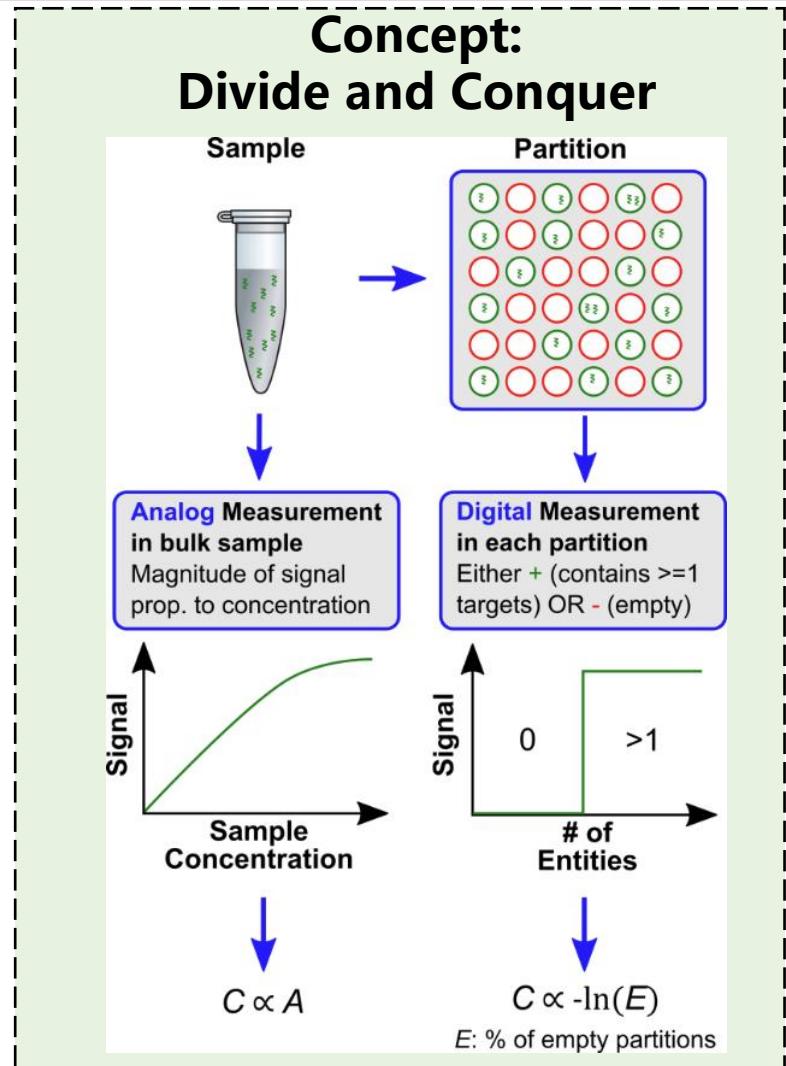


Detection Principles

Immunoassays: Enzyme-Linked Immunosorbent Assay (ELISA)

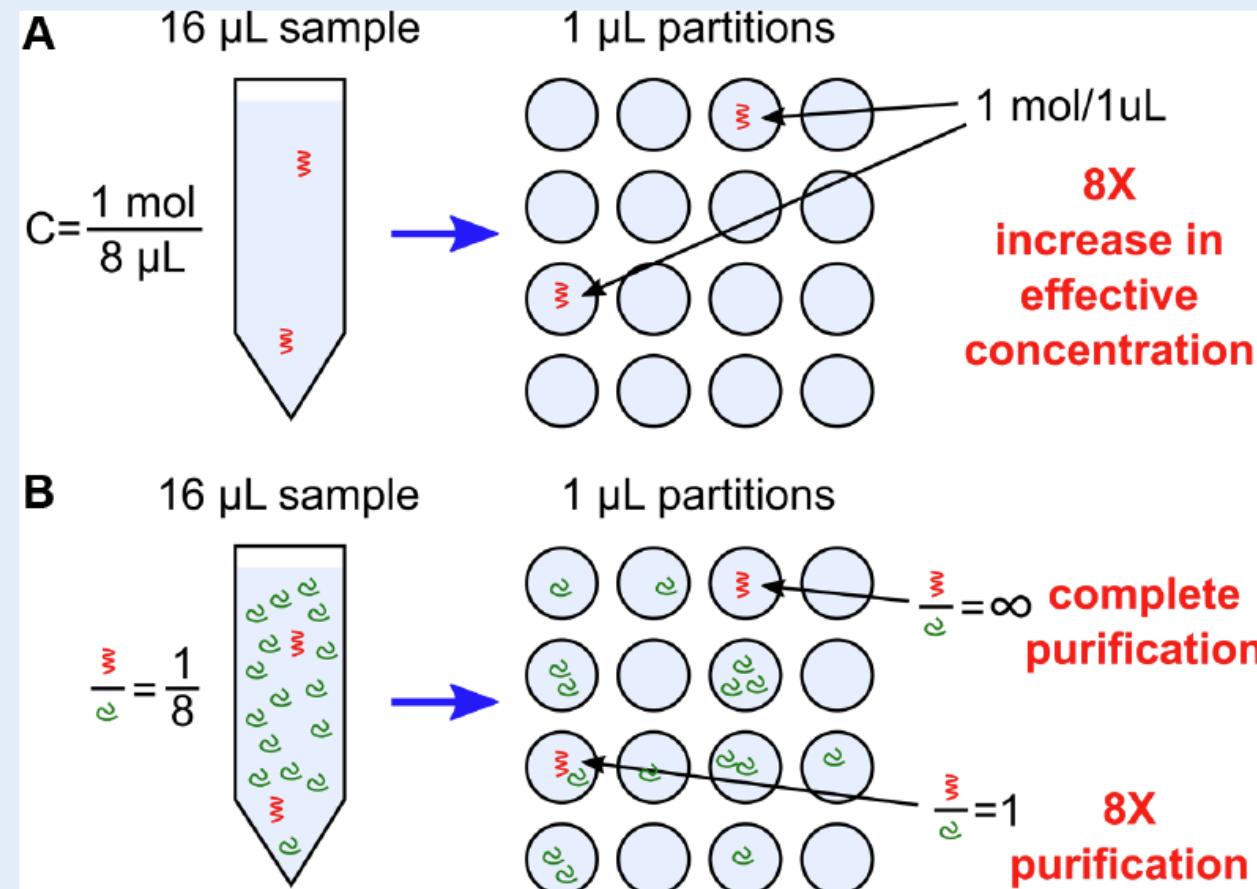


Digital Assay: Concepts and Advantages

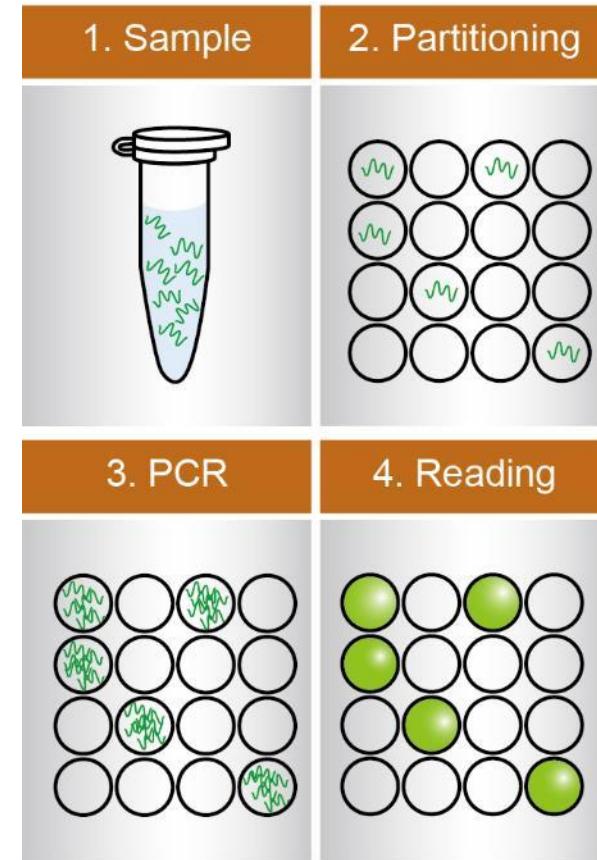
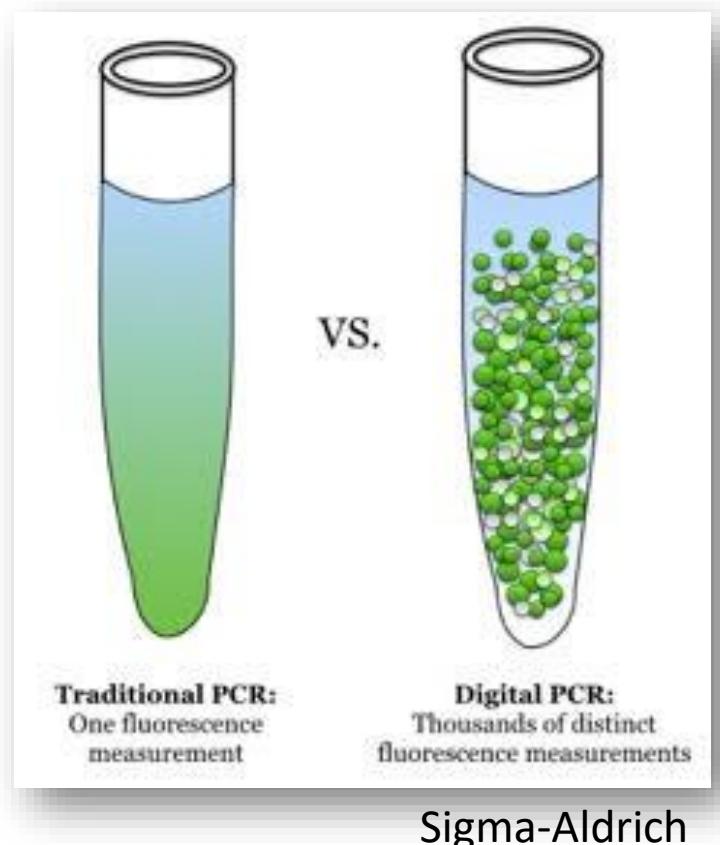


Digital Assay: Concepts and Advantages

Increase Effective Reaction Concentration; Enhance Target-to-Interference Ratio

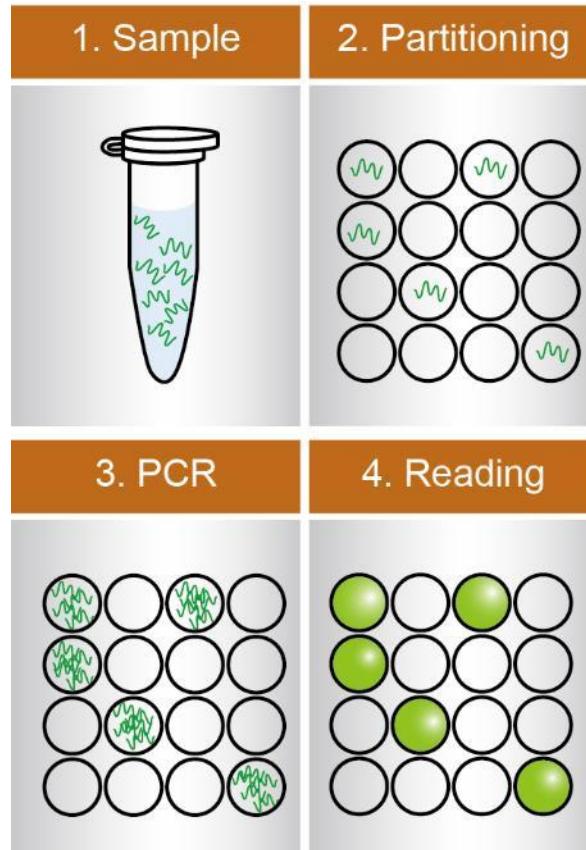


Principles of Digital PCR



Chen, Li,* et al. *Talanta*, 2021

Principles of Digital PCR



Chen, et al. *Talanta*, 2021

Poisson Distribution:
The probability of a droplet being empty is

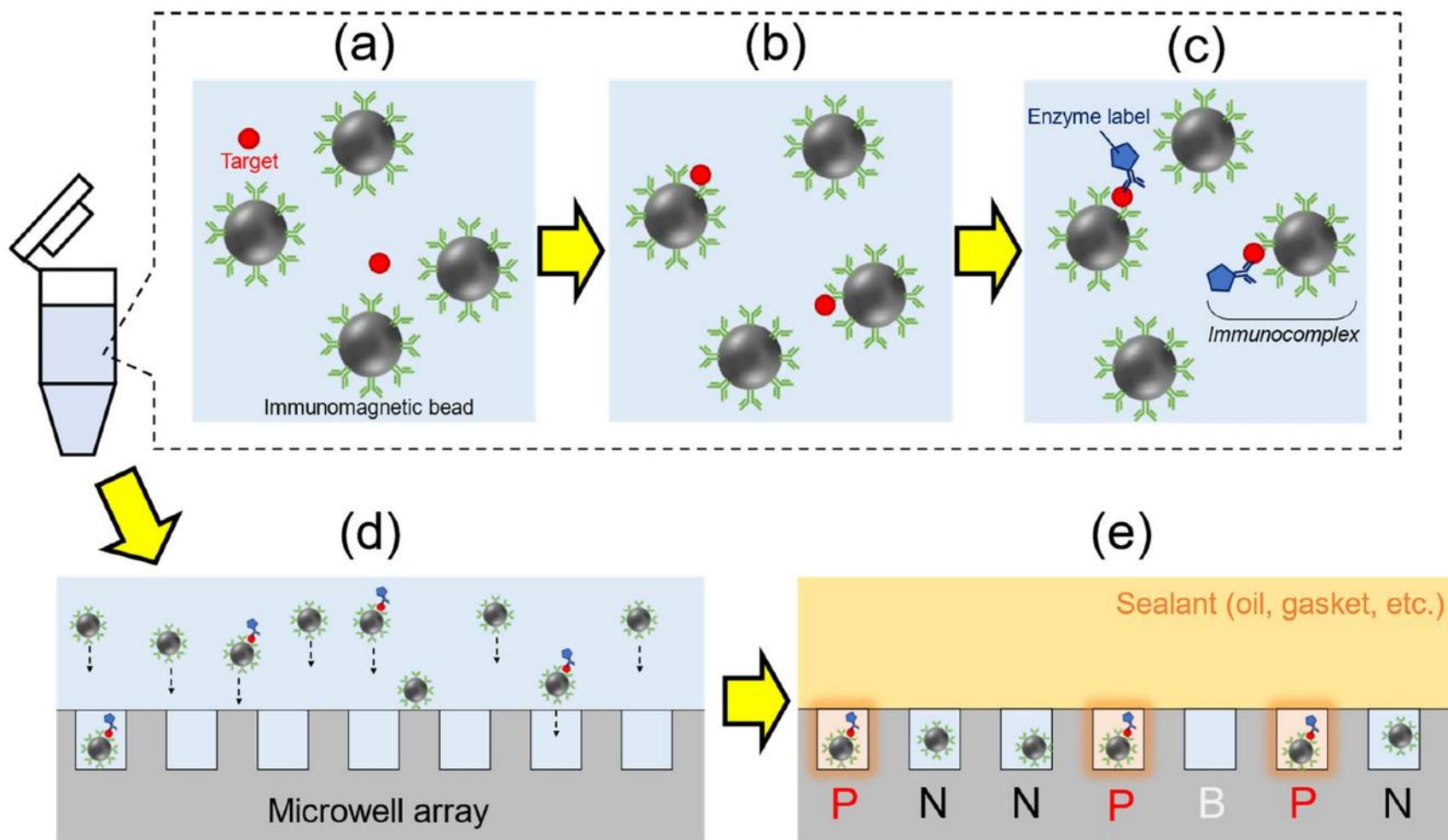
$$P^- = e^{-C \cdot V}$$

To infer the concentration:

$$C = -\ln P^- / V$$

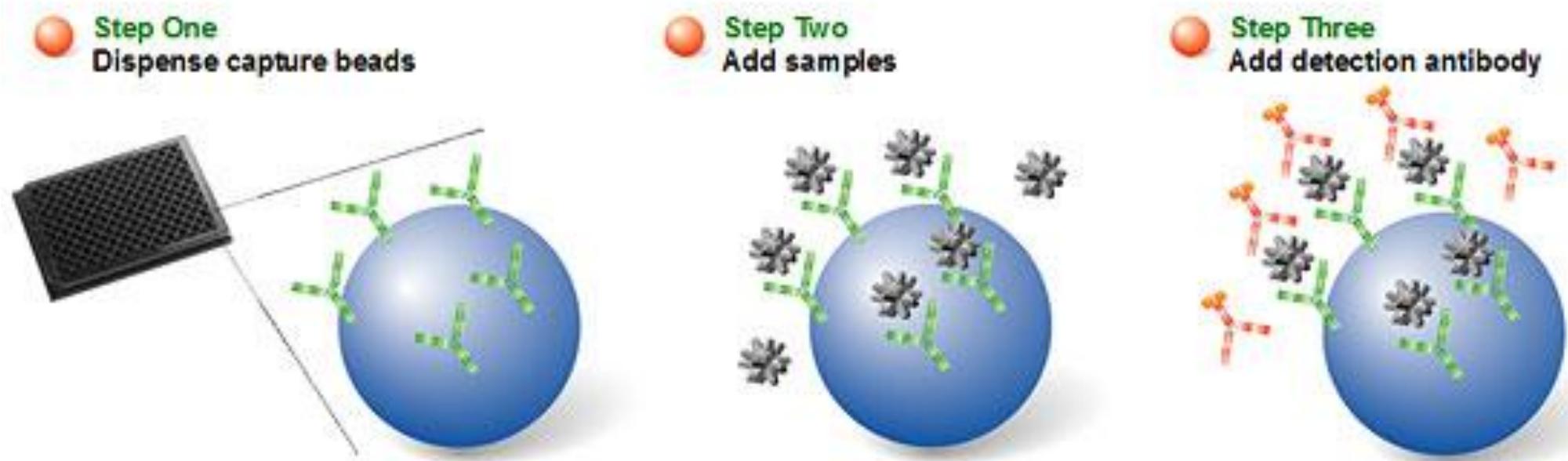
C : copies/ μL
 V : μL

Principles of Digital ELISA

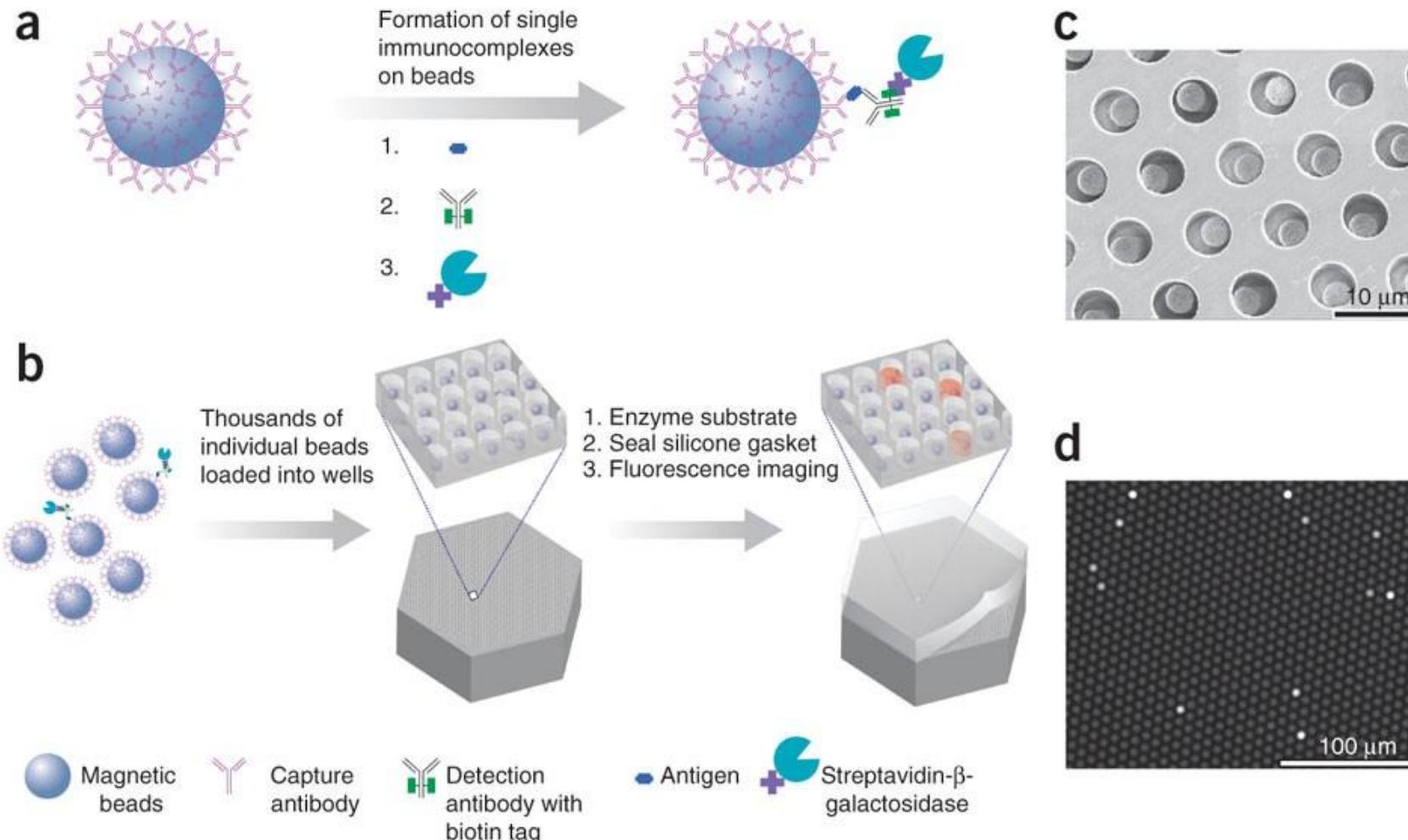


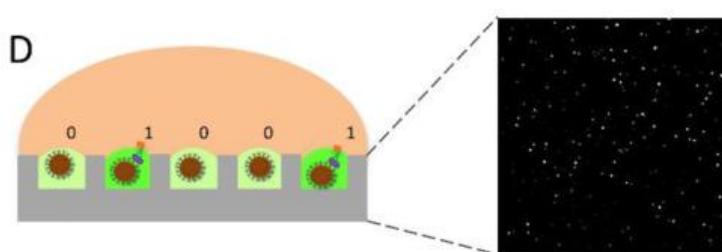
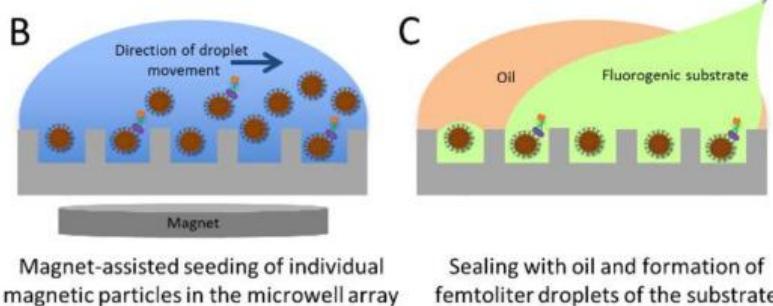
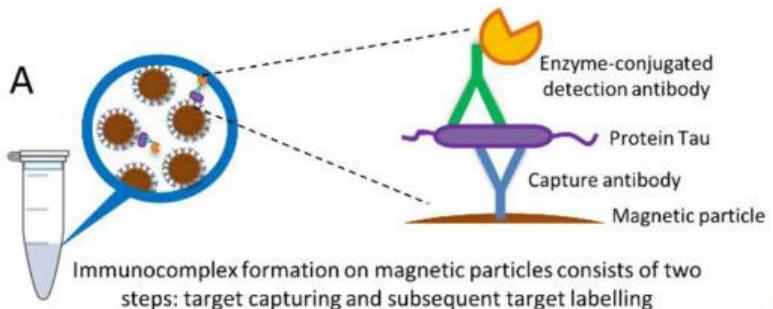
Part I: Digital Immunoassay

Generation of immunocomplex



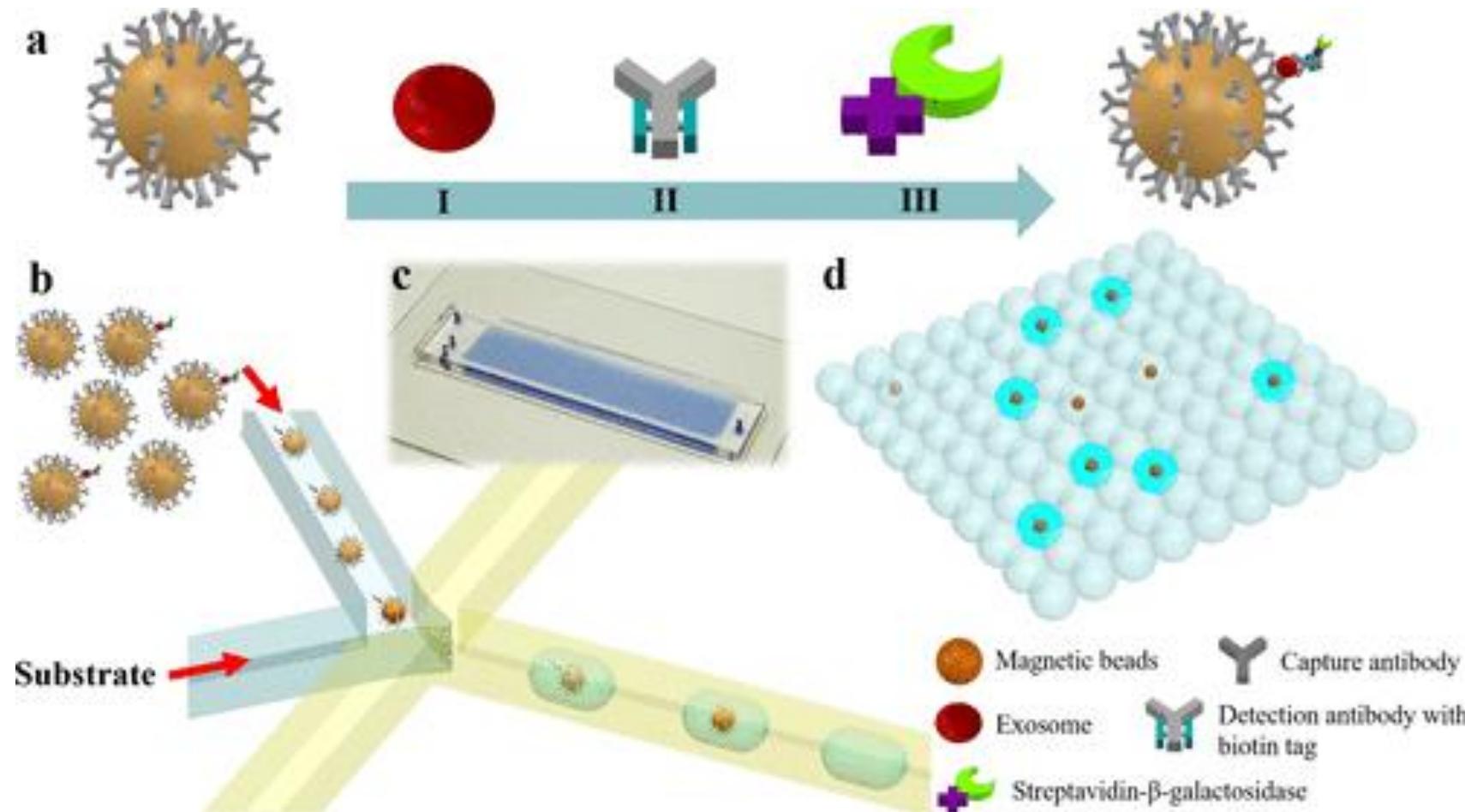
Microwell based dELISA

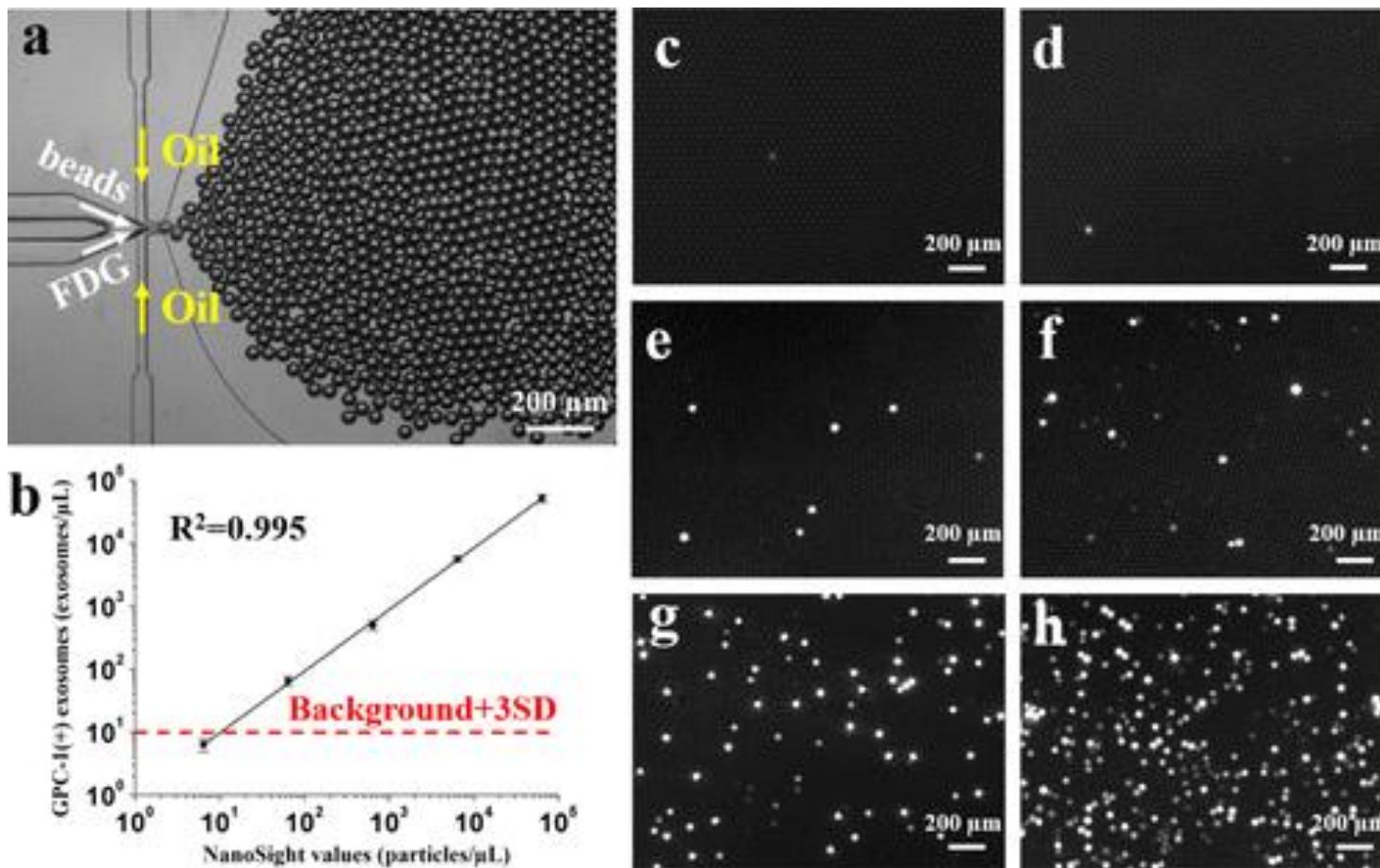




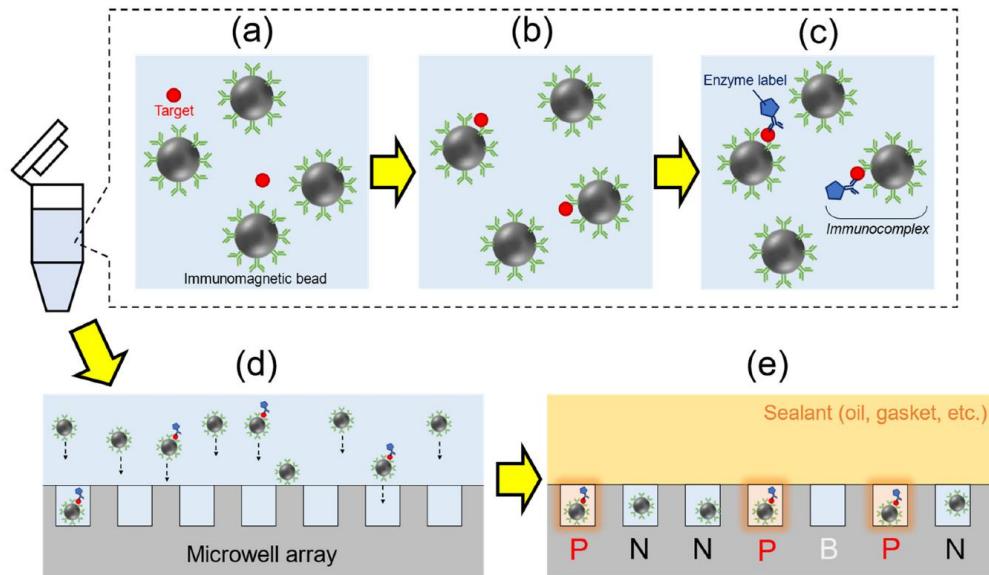
Incubating with fluorogenic substrate until generation of the fluorescent signal (left) and digital counting of single target protein molecules (right)

Droplet based



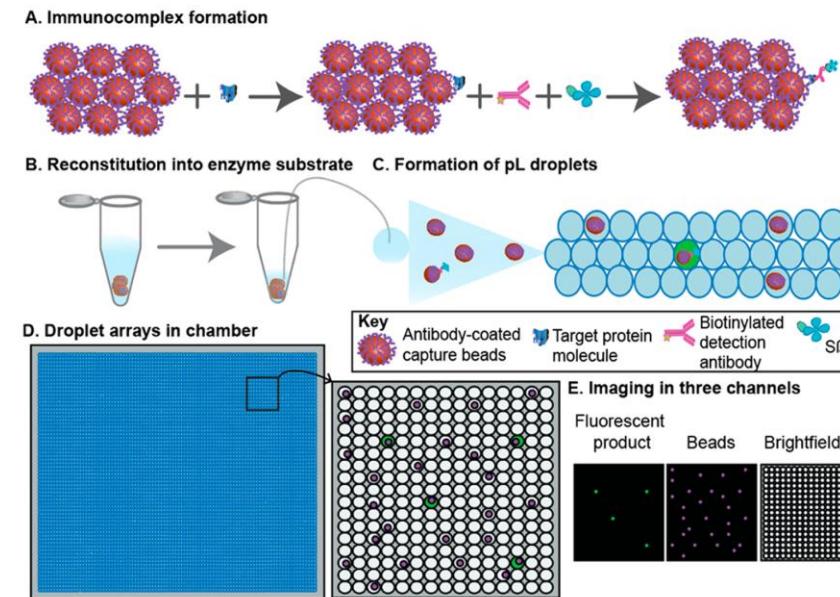


Microwell vs. Droplet Digital ELISA



Pros and cons:

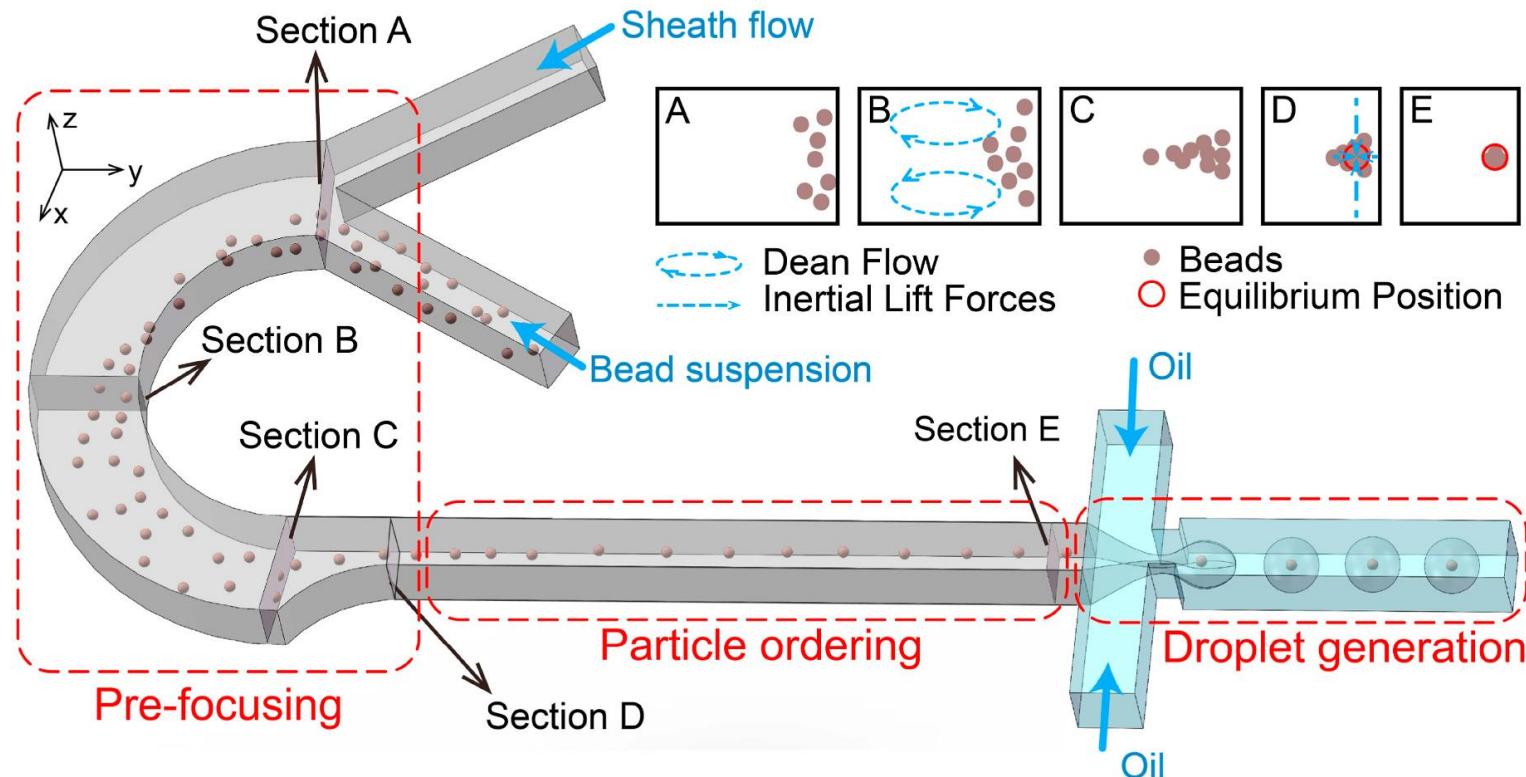
1. Easy to automate
2. Sampling Efficiency (Microsphere Recovery Rate): ~5%
 - Simoa: 500k \rightarrow 25k



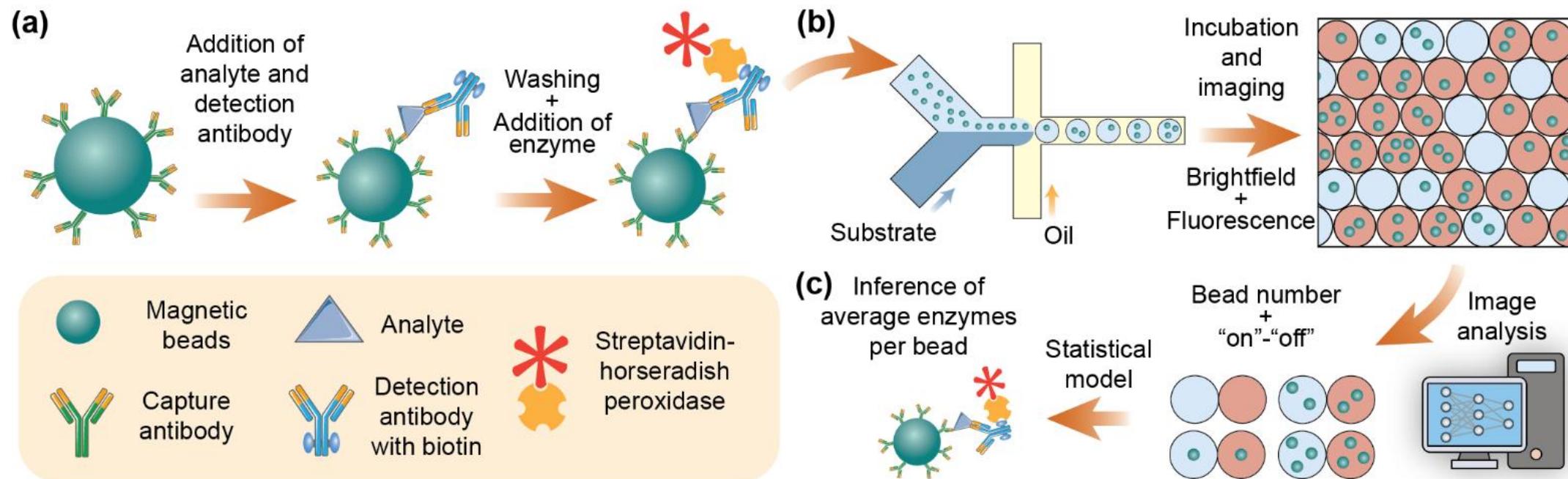
Pros and cons:

1. Sampling Efficiency (Microsphere Recovery Rate): ~60%
 - 100k \rightarrow 60k
2. Low Droplet Utilization Rate
 - 1000k \rightarrow 100k

Inertial Microfluidics Overcomes Poisson Distribution



Embracing Poisson Distribution



Analytical Chemistry, in revision

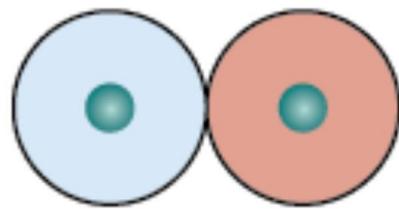
Different beads -> different on-off probability

λ : Average enzymes per bead

Bead contains at least one enzyme:

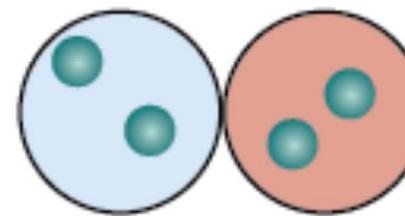
$$q = 1 - e^{-\lambda}$$

Bead being empty: $1-q$



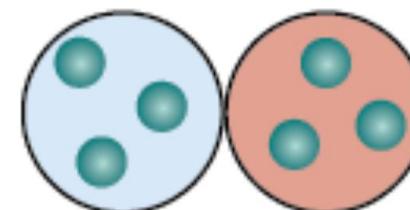
$$P_{1,-} = 1-q$$

$$P_{1,+} = 1 - P_{1,-}$$



$$P_{2,-} = (1-q)^2$$

$$P_{2,+} = 1 - P_{2,-}$$



$$P_{3,-} = (1-q)^3$$

$$P_{3,+} = 1 - P_{3,-}$$

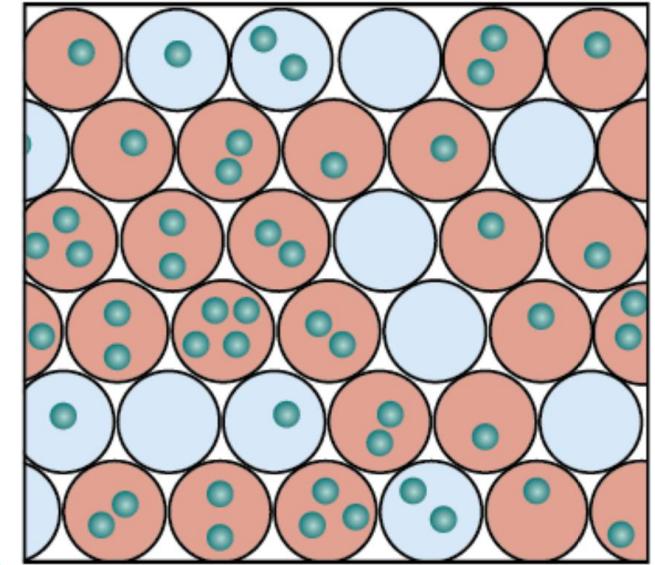
Maximum likelihood estimation

- For any observation (n_i, m_i)

$$L(\lambda; n_i, m_i) = \prod_{i=1}^{\infty} P_{i,+}^{n_i} \cdot P_{i,-}^{m_i}$$

- λ that maximizes this likelihood

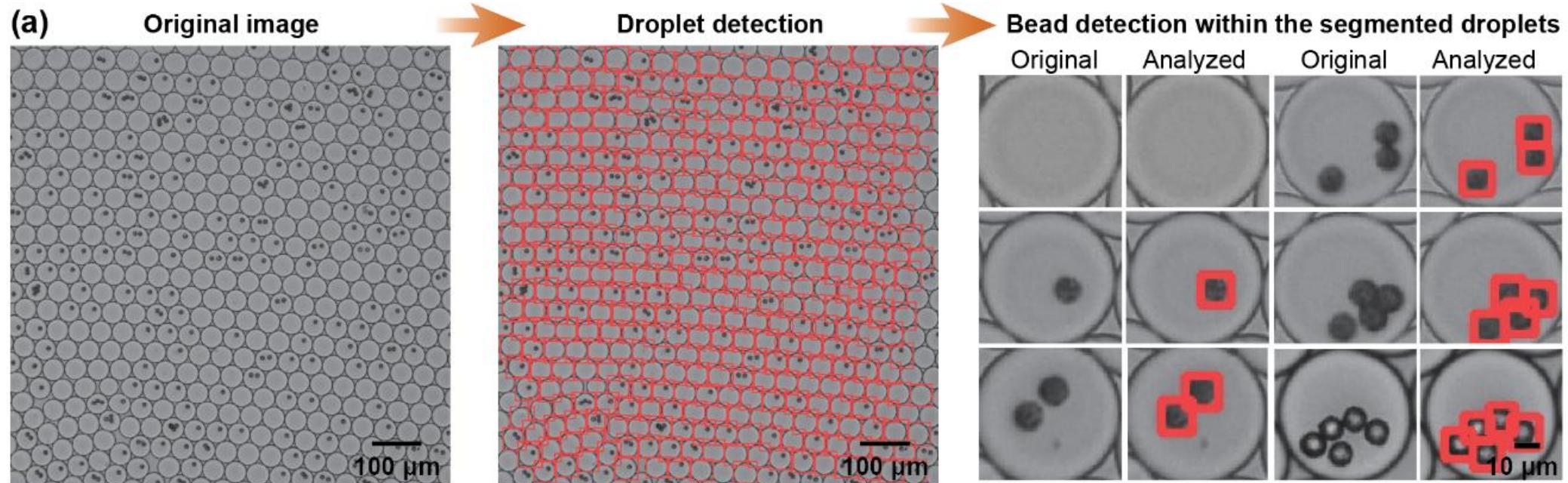
$$\hat{\lambda} = \arg \max \ln L(\lambda; n_i, m_i)$$



$$\frac{n_1}{1-(1-q)} + \frac{2n_2(1-q)}{1-(1-q)^2} + \frac{3n'_3(1-q)^2}{1-(1-q)^3} - \frac{m_1 + 2m_2 + 3m'_3}{1-q} = 0$$

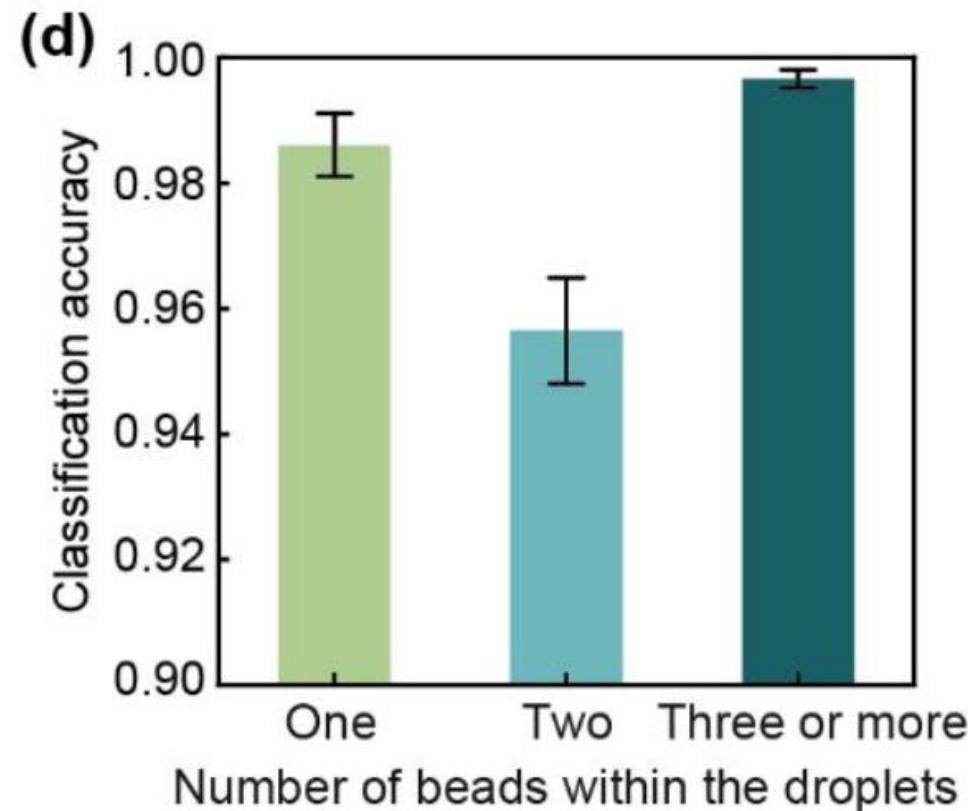
Droplet & bead detection

Deep learning image analysis: YOLOv5

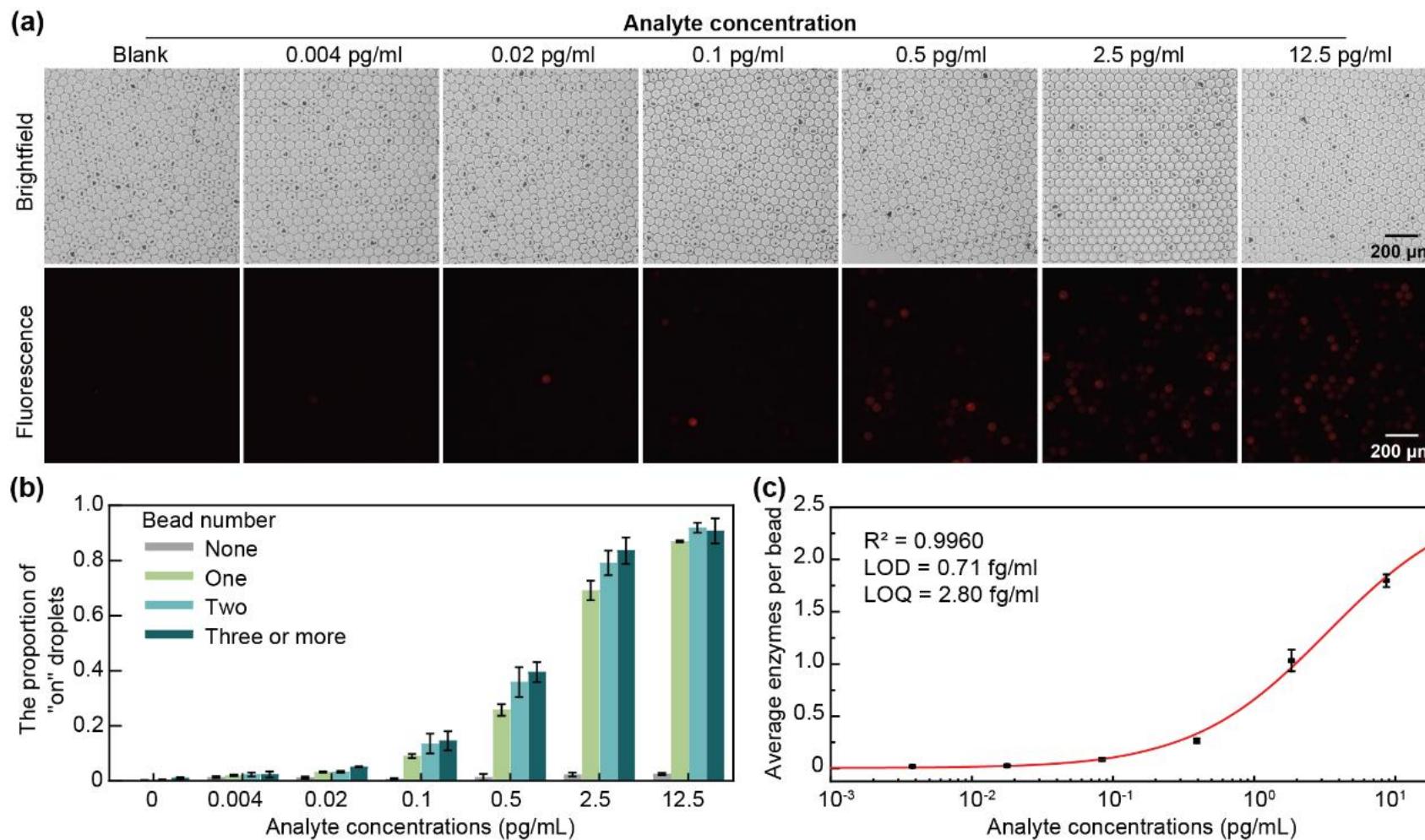


Droplet & bead detection

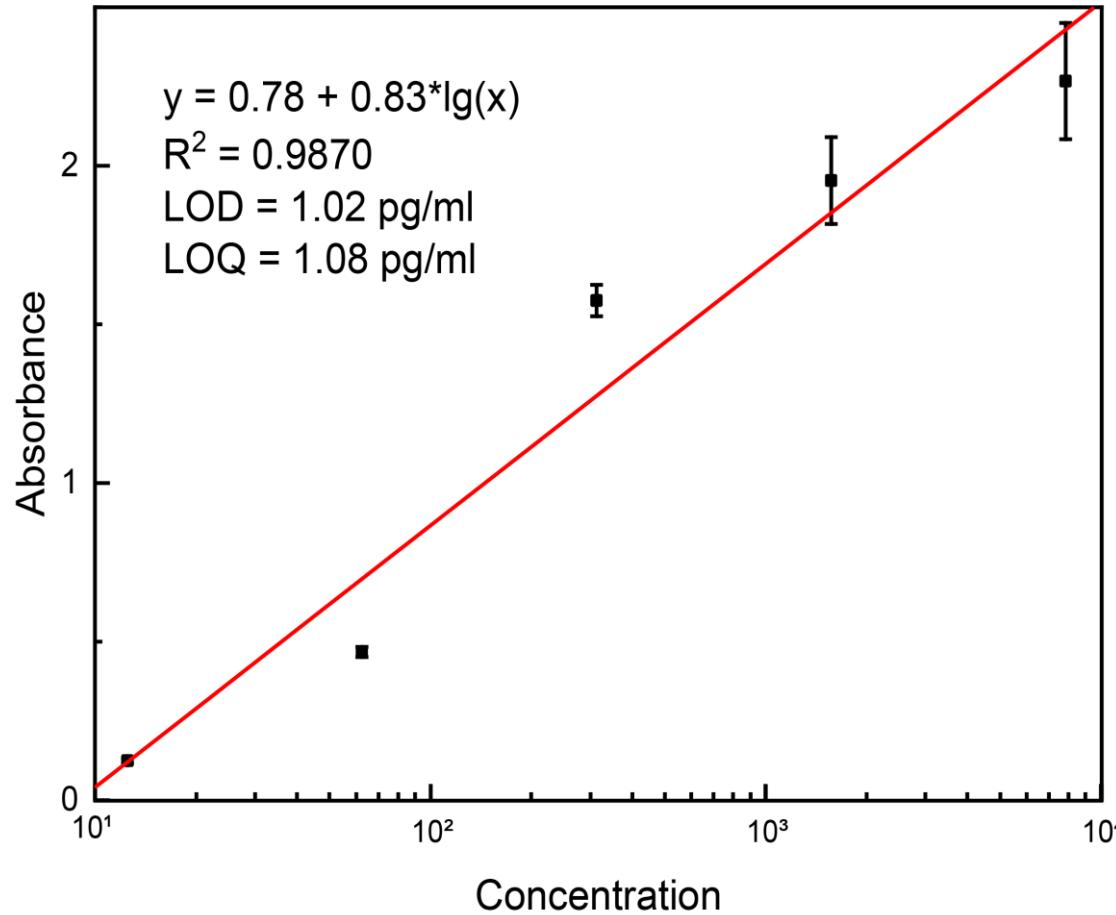
Deep learning image analysis: YOLOv5



Calibration

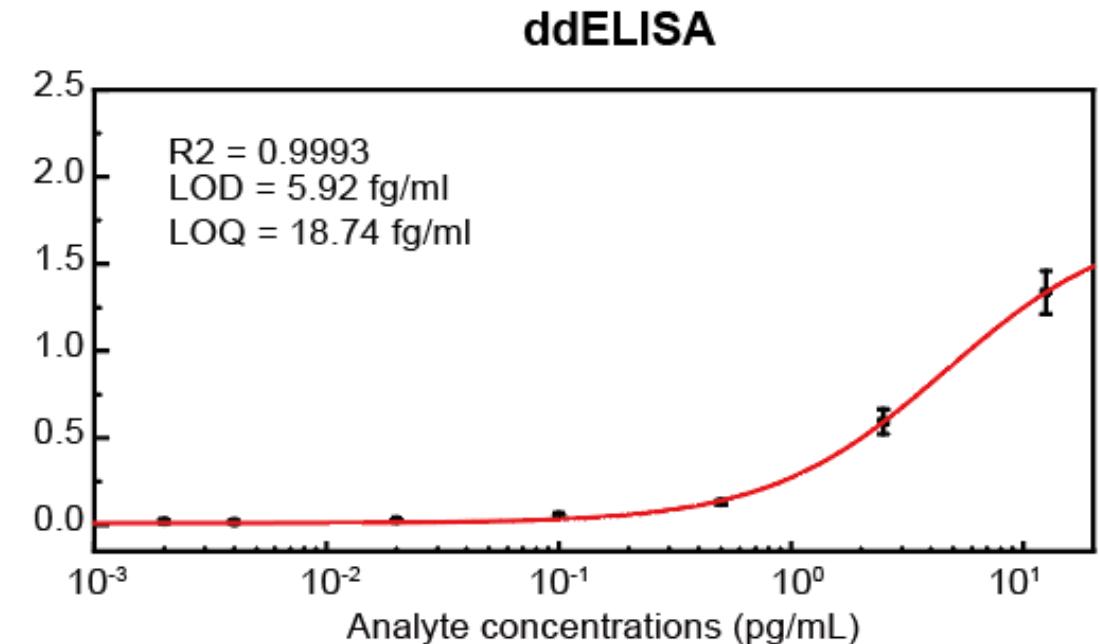
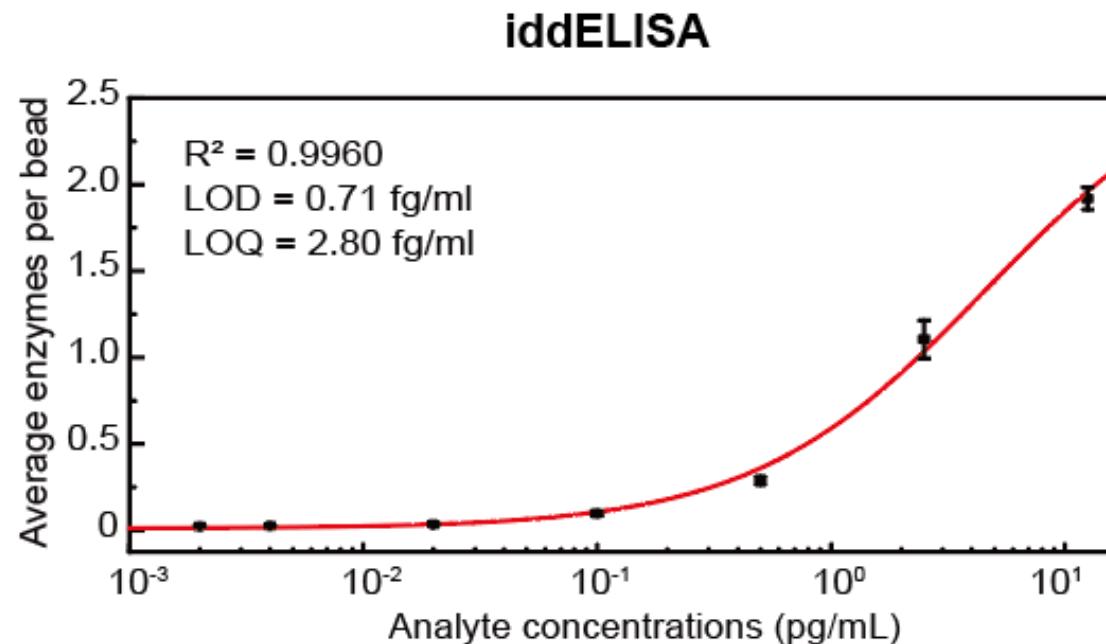


ELISA

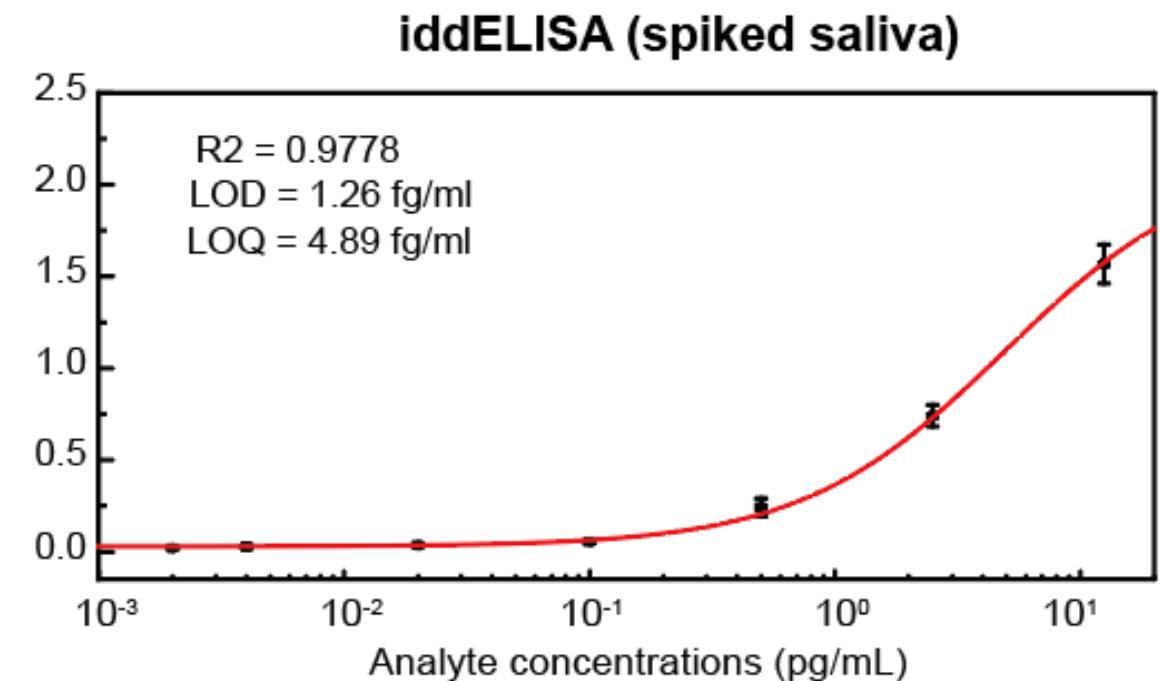
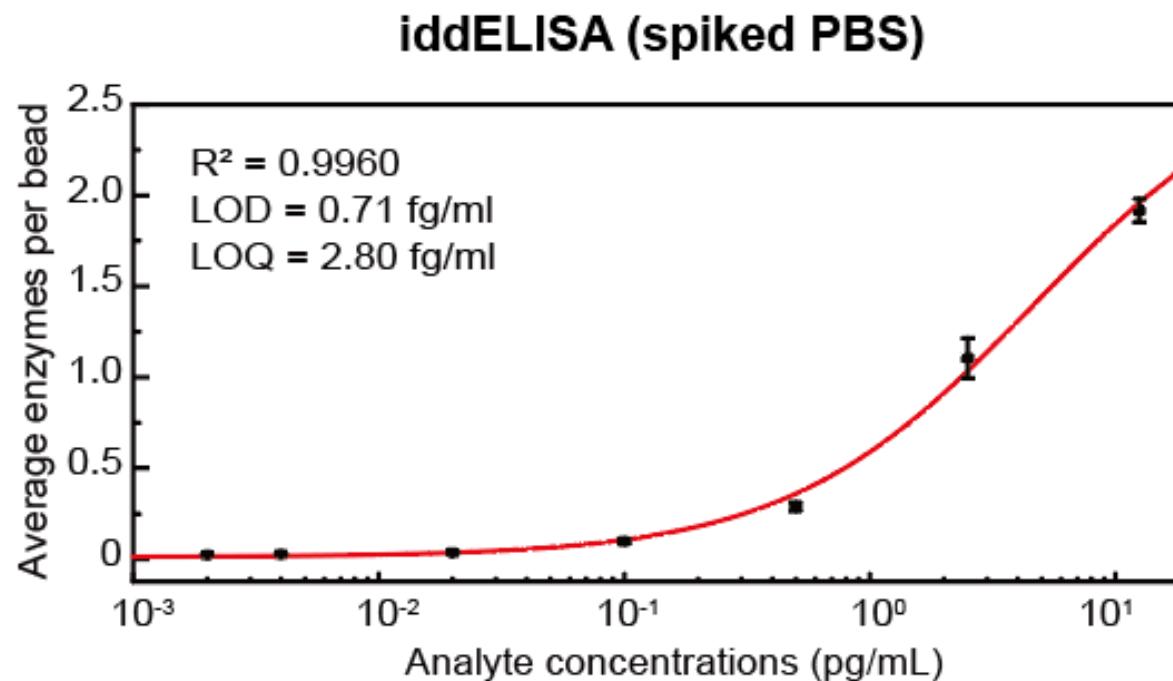


LOD:
0.71 fg/mL in iddELISA
1.02 pg/mL in ELISA

iddELISA vs. ddELISA



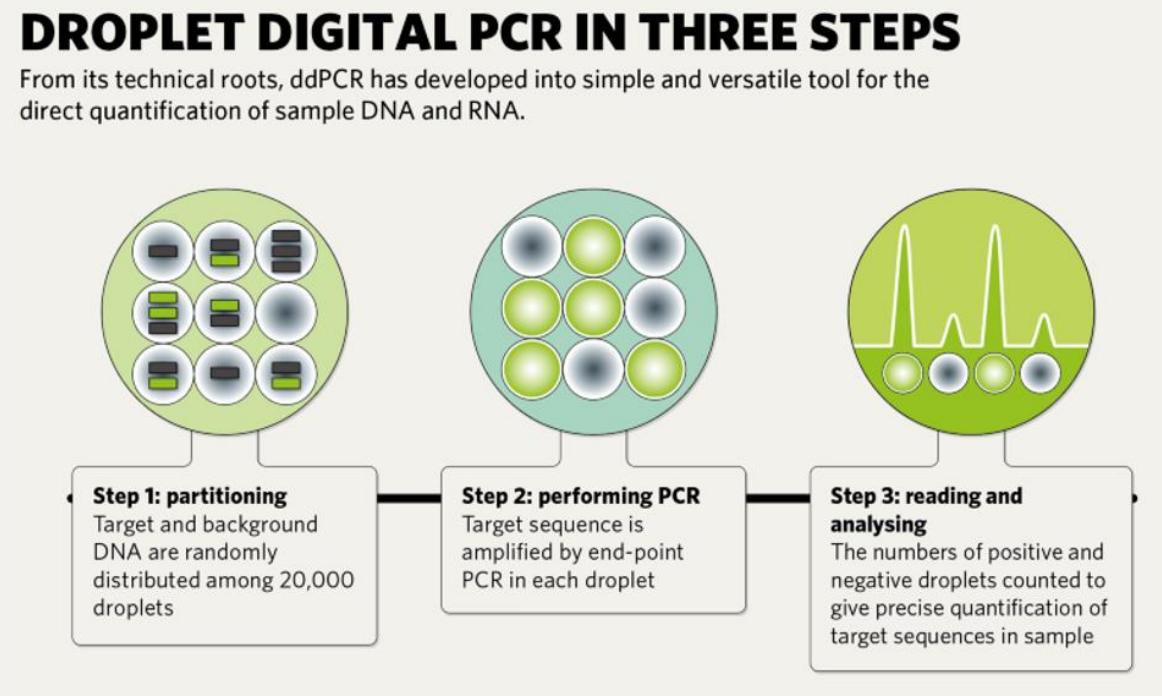
iddELISA in real sample



Multiplex digital ELISA

Part II: Digital Nucleic Acid Detection

Background: Digital PCR Requires Complex Equipment



Bio-Rad Laboratories

Droplet Generator

Droplet Reader



- **【Digital PCR Using the Bio-Rad QX100™ ddPCR™ System】**
<https://www.bilibili.com/video/BV1594y1q77y>
- **【Digital PCR Principle & Advantages】**
<https://www.bilibili.com/video/BV1Ey4y1G7JE>