HPAI diagnosis and laboratory biosafety

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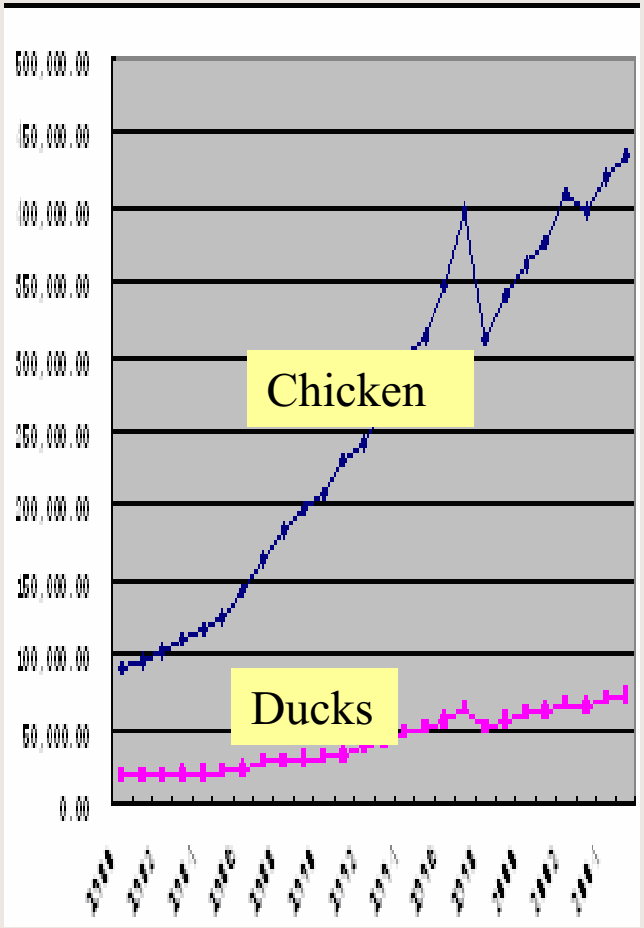
I. General Situation

China poultry annually increases by 3-8% for 3 decades

In 2005, China poultry:

- Total production: 14 billion
- Stocking population: 5 billion
- Chicken: 4.3 billion, 1/4 global



Infectious diseases emerge within this background

- Poultry production, transportation and live market increases so dramatically

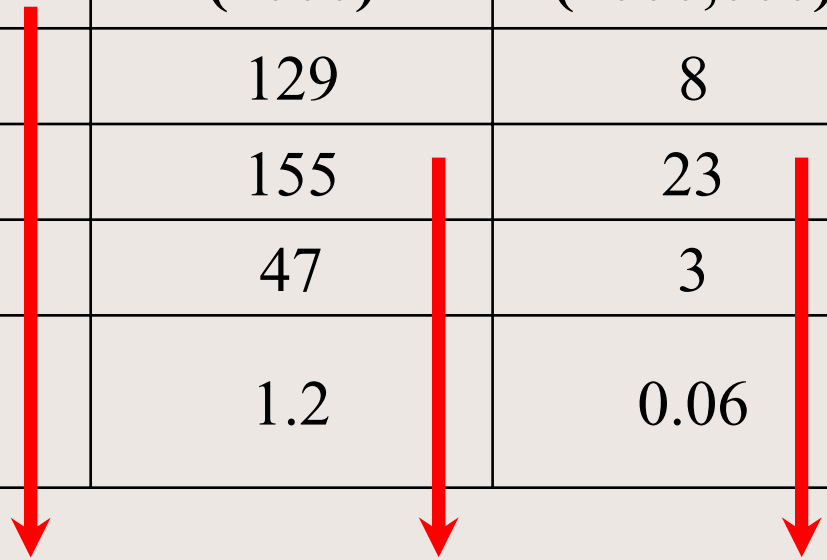
- Easy for infectious diseases to spread and circulate
- Difficult to control and eliminate infectious diseases

HPAI: 01/2004 - 08/2007

- 94 HPAI outbreaks confirmed in 22 provinces of China
- 34.6 million poultries culled
- 24 human cases from 2005, mainly in southern China.

Now, well controlled in China

YEAR	Outbreaks	Died (1000)	Slaughtered (1000,000)
2004	50	129	8
2005	32	155	23
2006	10	47	3
2007 >August	2	1.2	0.06



Principles: Early, Quick, Strict

- Early: Discover, diagnose, report, confirm ASAP
- Quick: Response ASAP
- Strict: Strict measures

Contain the outbreak

Minimize the spread

Vaccination & Stamping-out Policy

- Vaccination: all domestic birds are compulsory for vaccination, even for backyard flocks.
- Stamping-out: all the infected and suspect animals within the epidemic spot should be slaughtered, well buried and disinfected.

Ⅱ. Lab Biosafety of HPAI

Who benefits from biosafety?

- **Farmers (direct)**
- **Government (indirect)**

  More tax income and less expenditure

Lab Biosafety Principle 1

Double guarantee

- Containment (Keep pathogens in)
- Disinfection (Kill pathogens)

1. Containment

Keep pathogens in

- The tips
- The tubes
- The bottles
- The boxes
- The rooms
- The buildings

2. Disinfection

- **Physical:** **Boil; Autoclave; Ultraviolet; ……**
- **Chemical:** **Acid; Alkaline; Ethanol; ……**

Lab Biosafety Principle 2

4 components:

- **Recognition**
- **Management**
- **Behaviors**
- **Facilities**

Example 1: Africa

Example 2: Hong Kong

Minimum requirements

- **Nose mask**
- **Clothes**
- **Gloves**
- **Boots**
- **Wash hands**

Two basic concepts

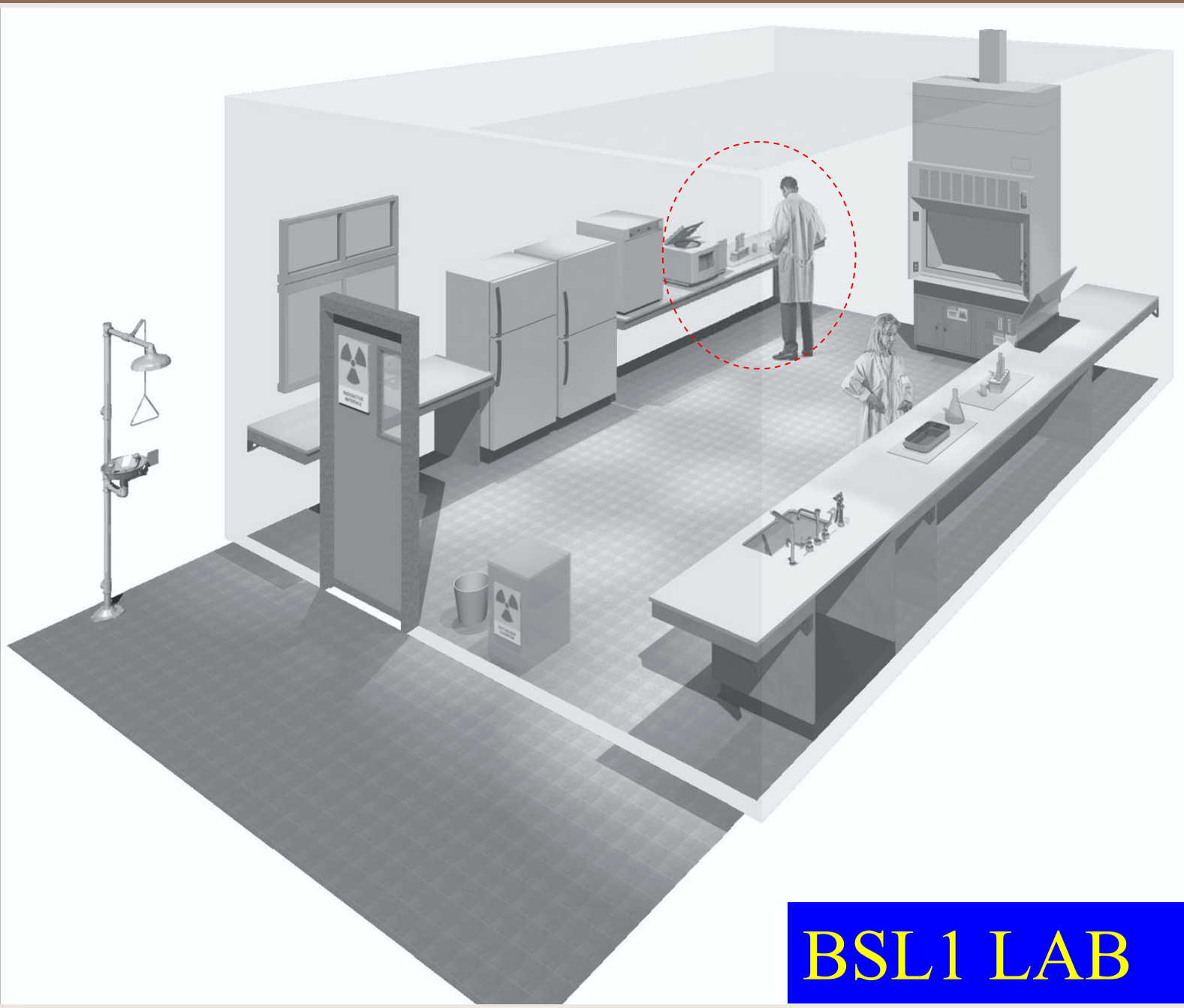
- Risk Classification of pathogens
- Lab Biosafety Levels

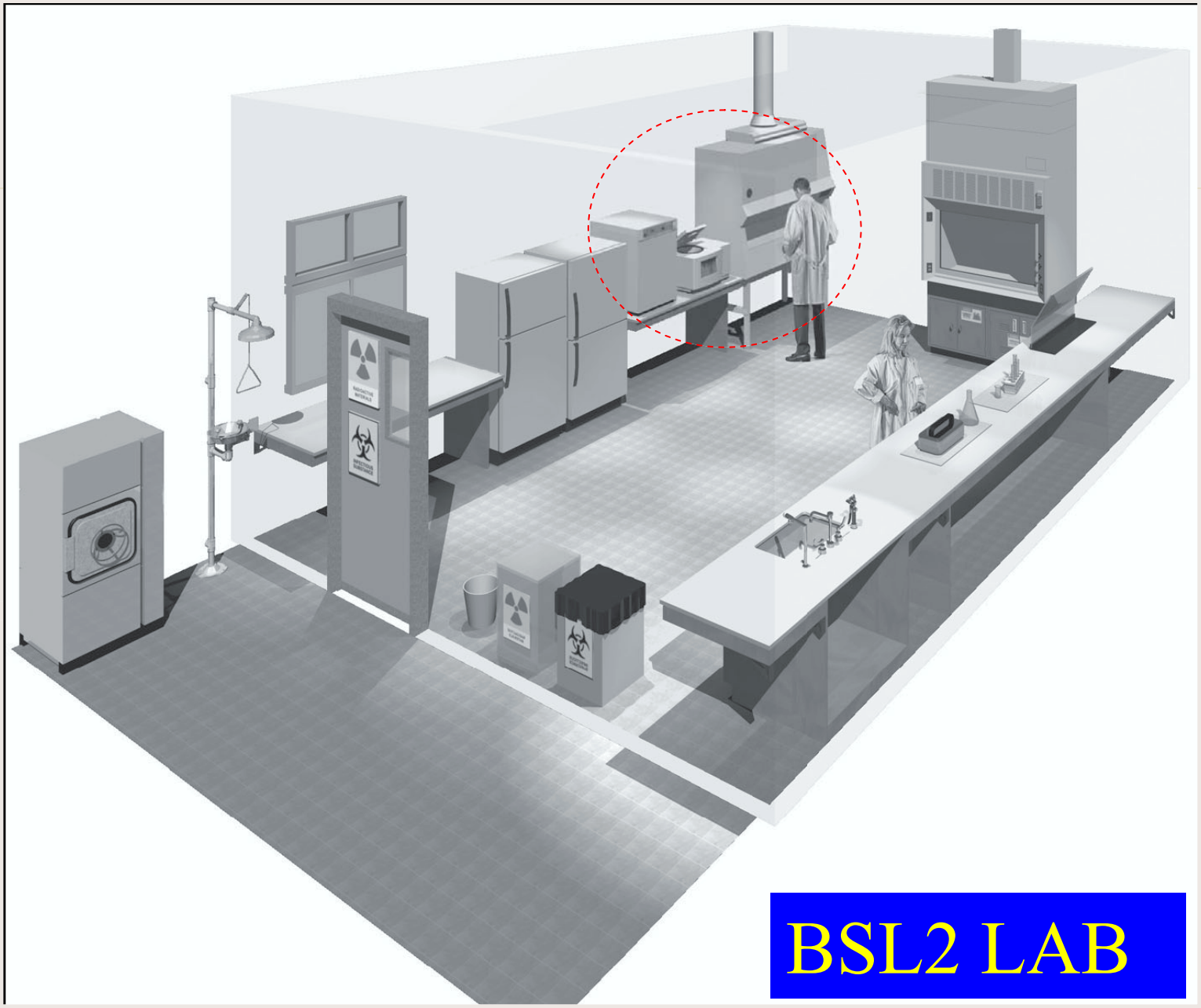
1. Risk Classification of pathogens

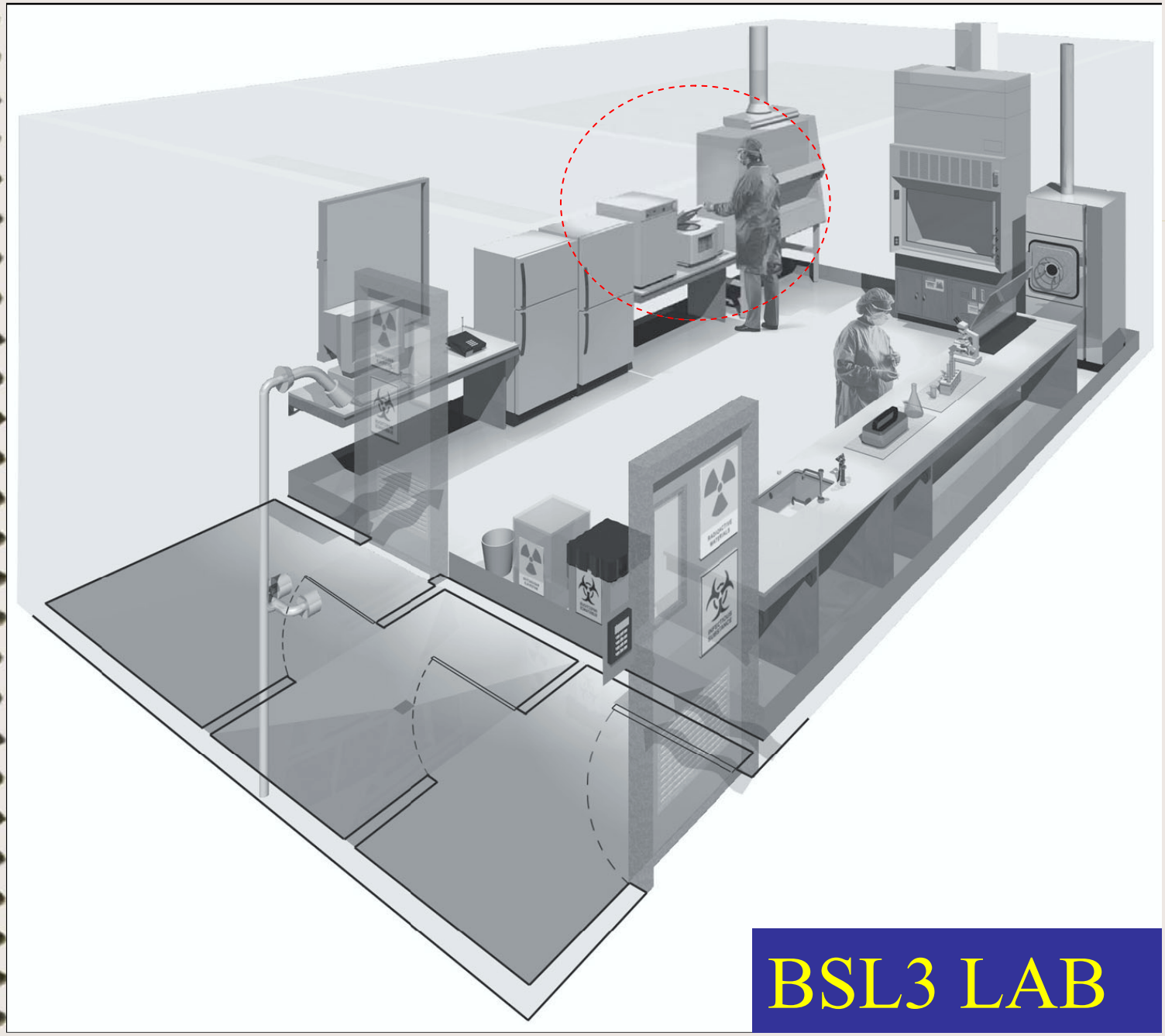
Risk Group	Individual risk	Community risk
1	low	low
2	moderate	low
3	high	low
4	high	high

2. Lab Biosafety Levels (BSL)

- Basic – BSL1, BSL2
- Containment – BSL3
- Maximum containment – BSL4


BSL1 LAB


BSL2 LAB



BSL3 LAB

BSL4 LAB

For more details:

Google search

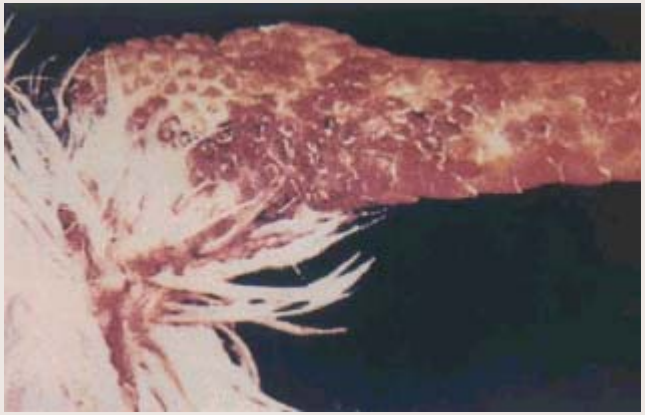
Minimum requirements in the lab

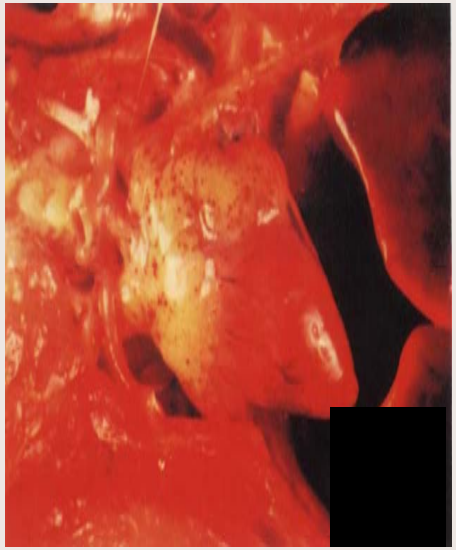
- **Healthy**
- **Nose mask and protection clothes**
- **Wash hands**
- **Autoclave clinical samples and inoculated embryos before disposal**

Ⅲ. Diagnosis overview

Clinical Diagnosis

- Sudden a lot of deaths
- Hemorrhage: multiple organs
- Especially: hock, brain and heart
- Difficult to differentiate from Newcastle disease virus infection





2 kinds of laboratory diagnosis

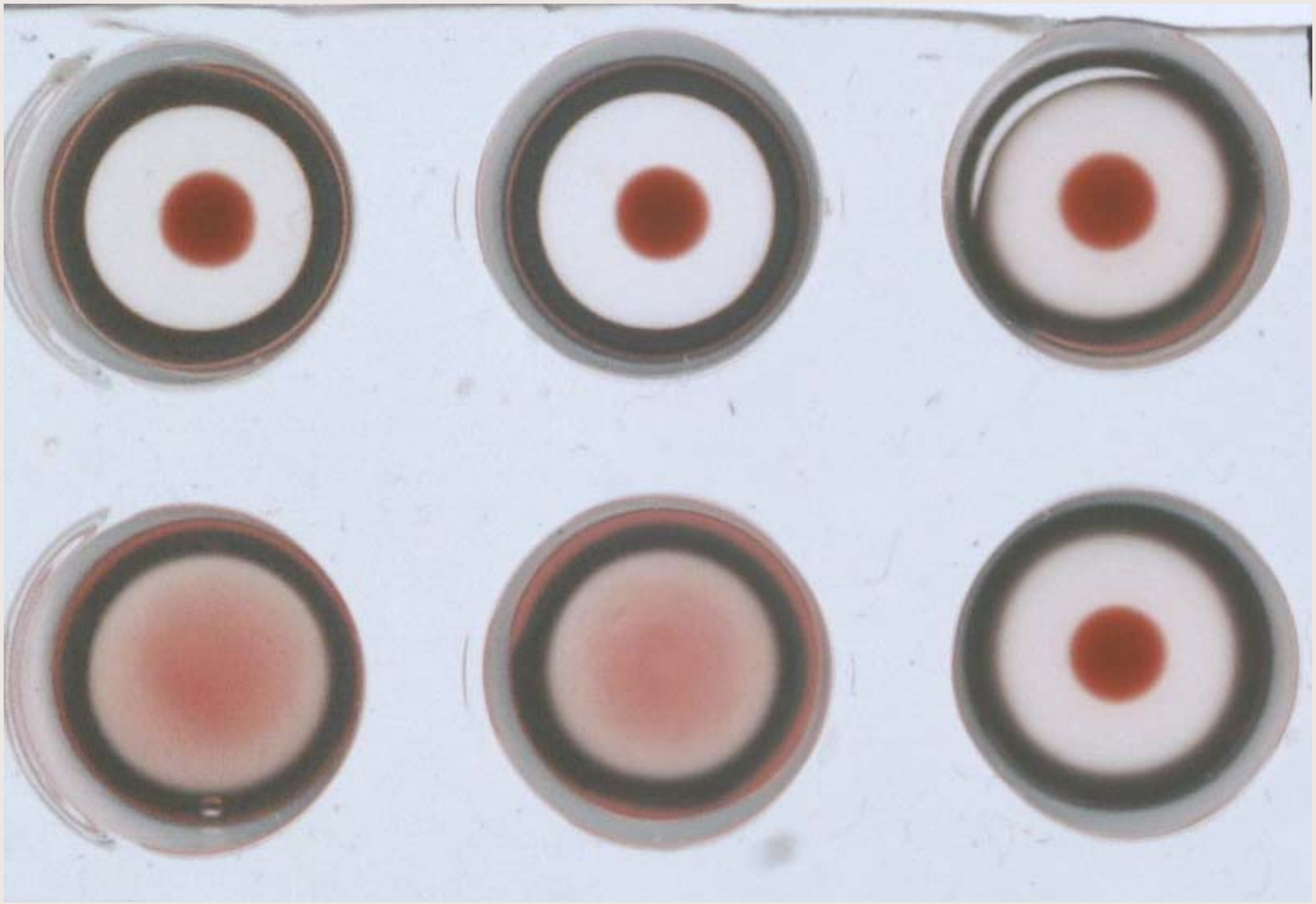
- Antibody detection: infected or vaccinated
- Pathogen detection: clinical diagnosis and outbreak confirmation

Antibody detection method

- Haemagglutination test (HA test)
- Haemagglutination inhibition test (HI test)

HA/HI test

- AIV has haemagglutination (HA) property
- Some other viruses, like NDV, also have HA property



- The HA property of H5 subtype AIV can be inhibited by the antibodies specific to H5 subtype AIV, but can not by the antibodies specific to H9 subtype AIV
- HA/HI can be used to detect unknown antibodies using standard pathogens
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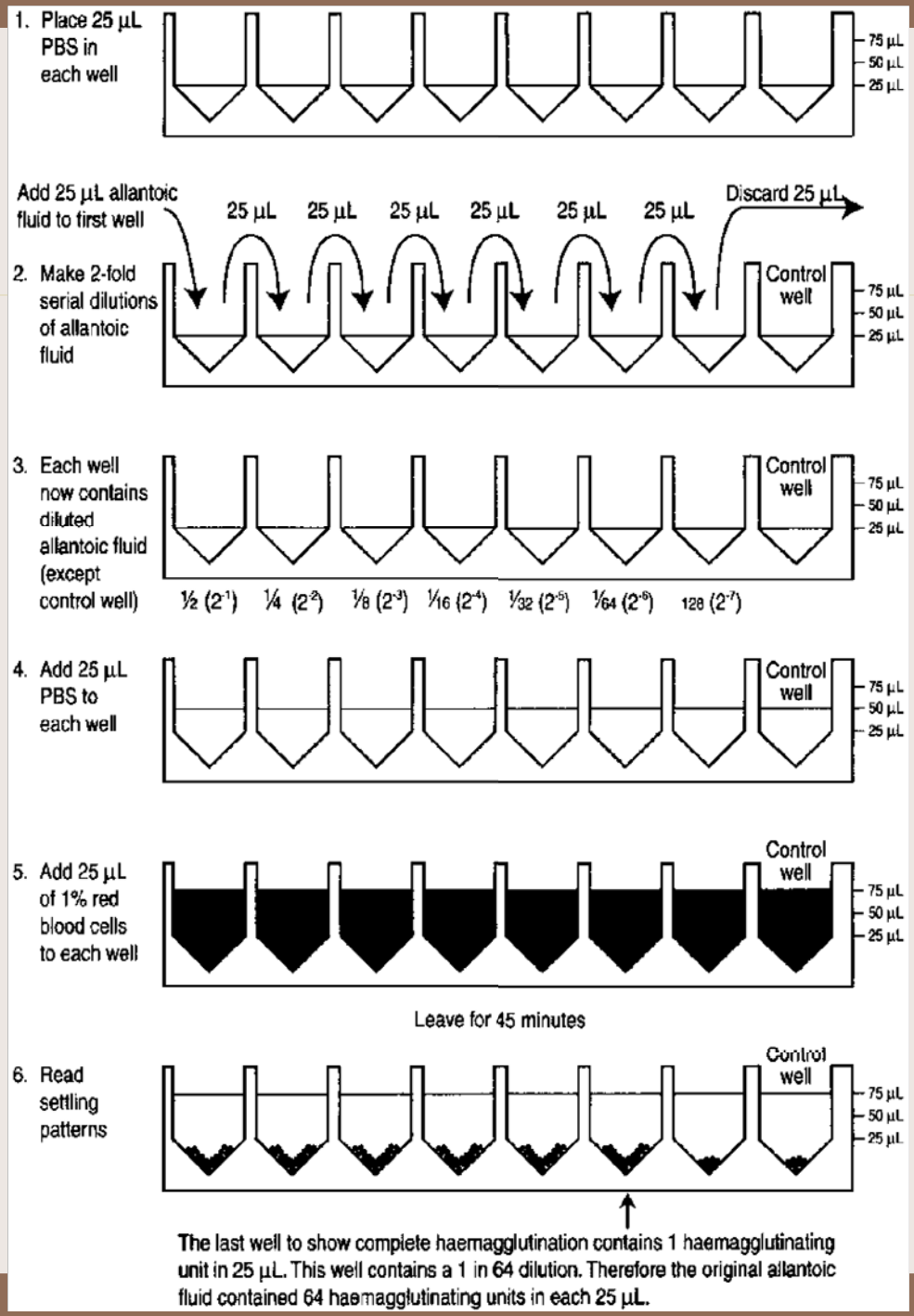

1. Place 25 µL PBS in each well
75 µL
50 µL
25 µL
Add 25 µL allantoic fluid to first well
25 µL
25 µL
25 µL
25 µL
25 µL
25 µL
Discard 25 µL
2. Make 2-fold serial dilutions of allantoic fluid
Control well
3. Each well now contains diluted allantoic fluid (except control well)
½ (2-1)
¼ (2-2)
⅛ (2-3)
1/16 (2-4)
1/32 (2-5)
1/64 (2-6)
128 (2-7)
4. Add 25 µL PBS to each well
5. Add 25 µL of 1% red blood cells to each well
Leave for 45 minutes
6. Read settling patterns
The last well to show complete haemagglutination contains 1 haemagglutinating unit in 25 µL. This well contains a 1 in 64 dilution. Therefore the original allantoic fluid contained 64 haemagglutinating units in each 25 µL.

Pathogen detection methods

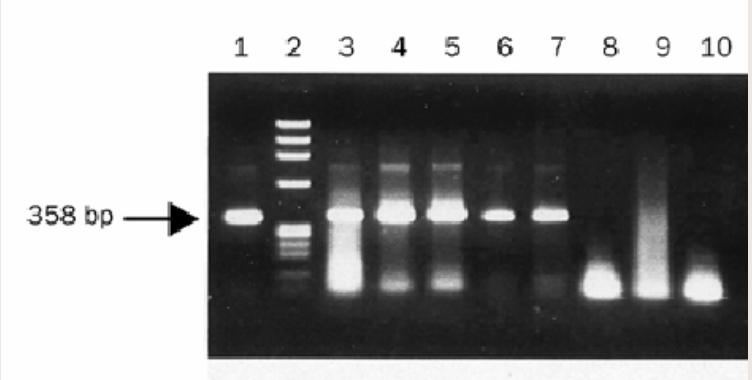
- Virus isolation followed with HA/HI
- RT-PCR to detect the viral nucleic acid
- Pen-side kit to detect the viral protein

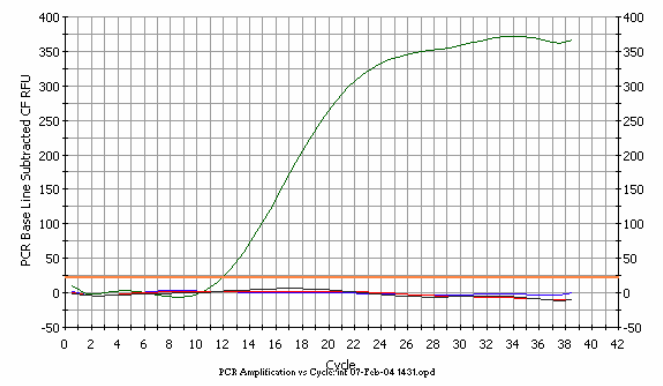
1. Virus isolation

- Using SPF or AIV-unvaccinated chicken embryos
- Using throat or cloacal swabs
- Detect the virus using HA/HI test after 4-day incubation

2. RT-PCR

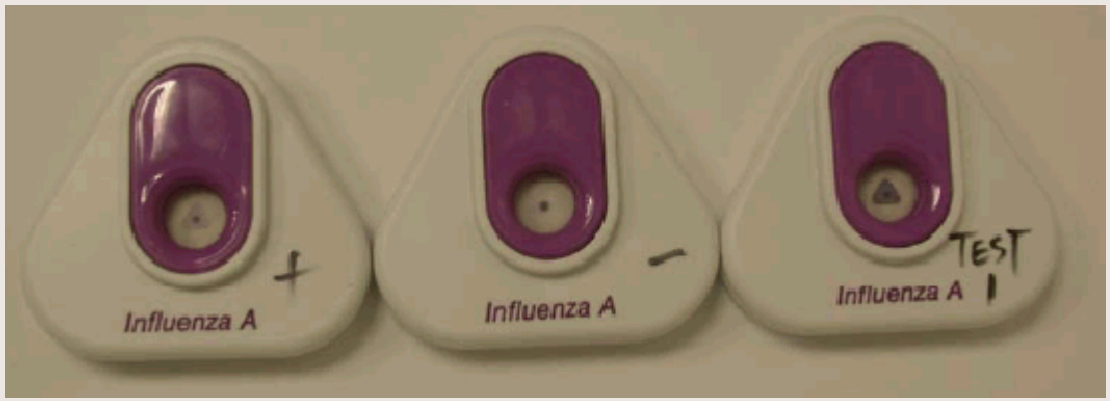
- Viral RNA can be amplified many times through RT-PCR
- RT-PCR specific to all subtype
- RT-PCR specific to H5 subtype





3. Pen-side kit

- Immunoassay on a filter
- Quick, simple, but not sensitive enough



For more details:

- *Manual for the laboratory diagnosis of Avian Influenza*

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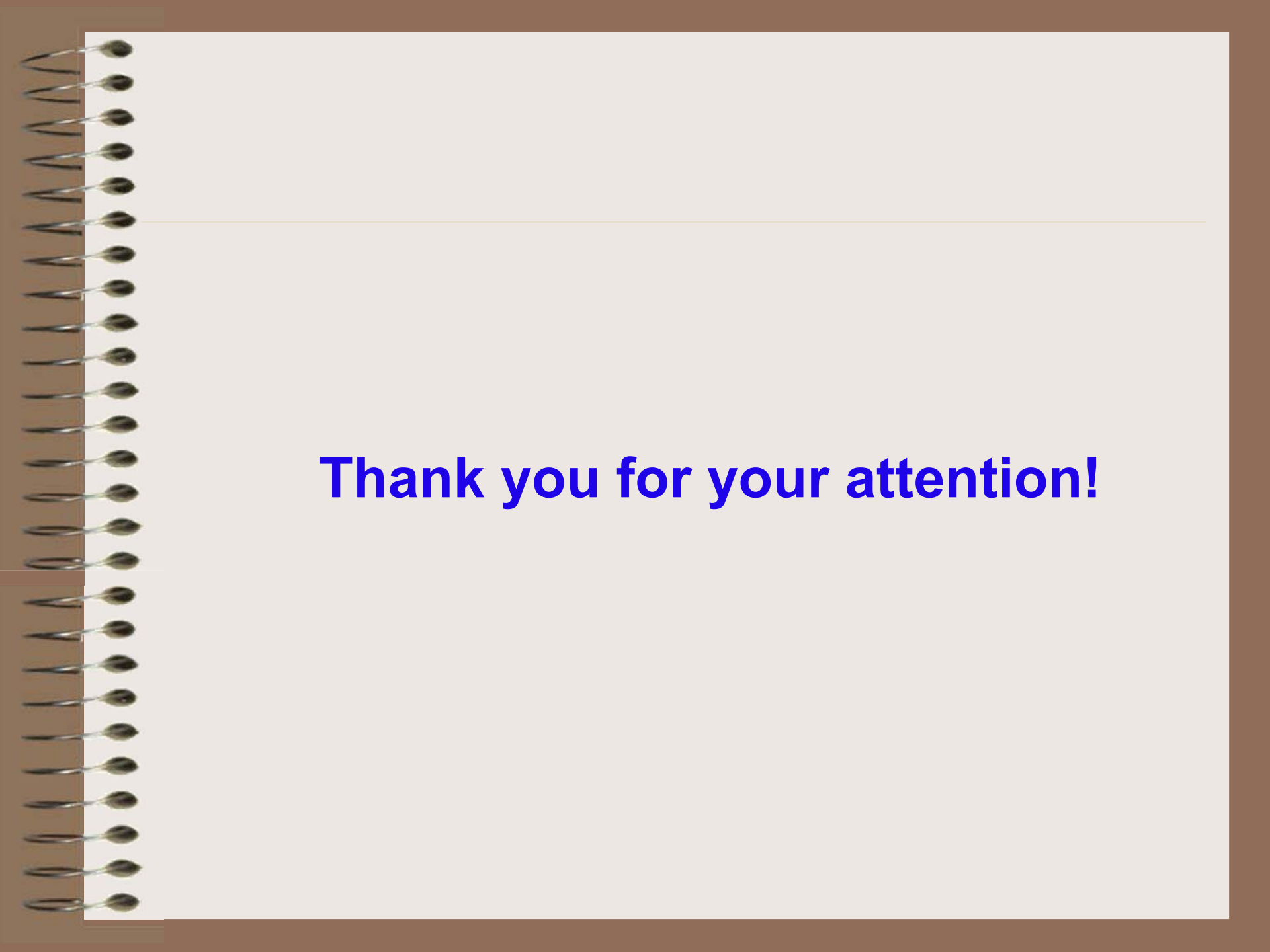
Thank you for your attention!