The Shock of the New:

Addressing the Challenges of New Technologies and Microbiological Monitoring

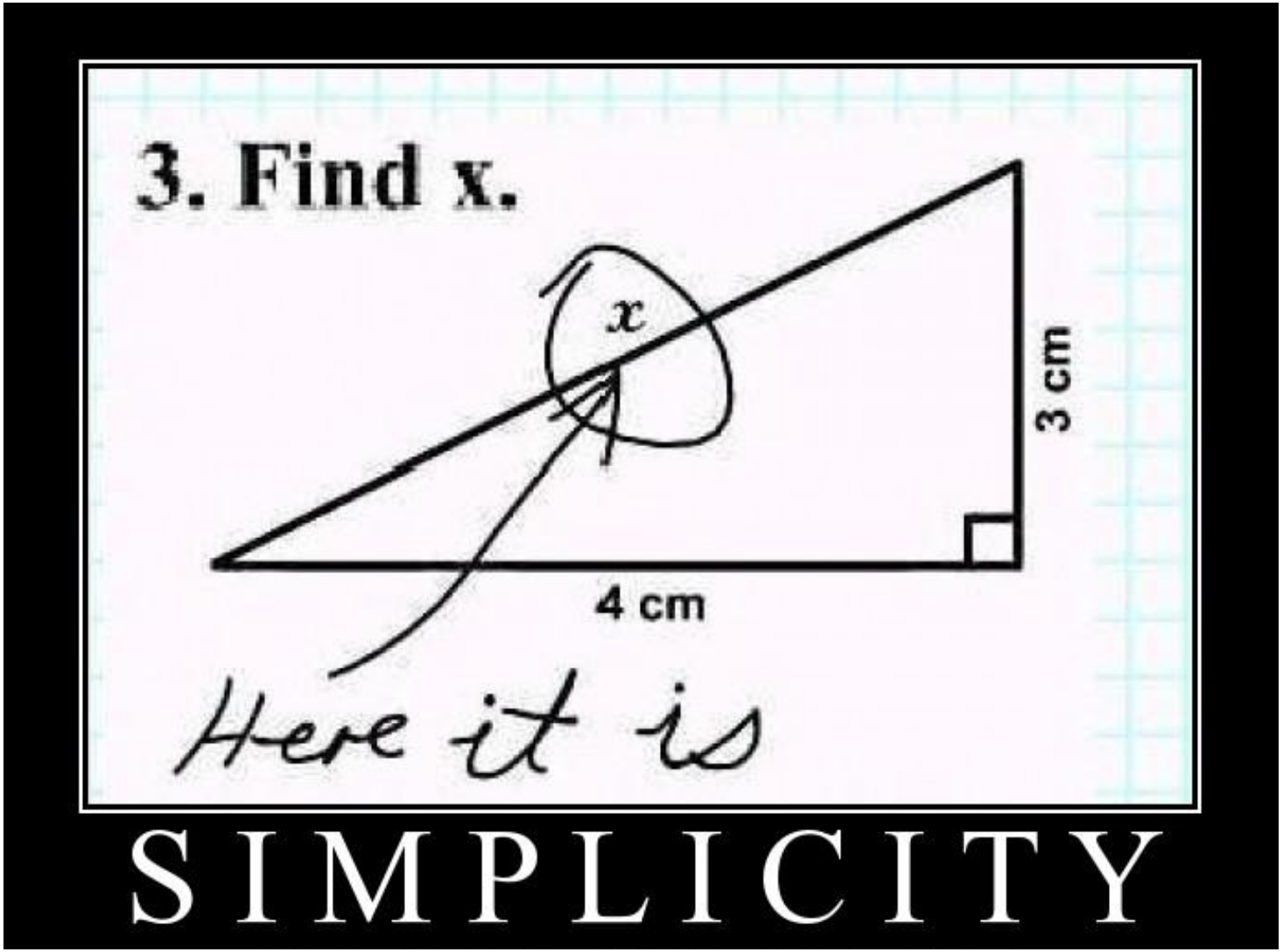
Adrian Deeny

University College London

Summary

- Why health monitoring is important
- The aims of health monitoring
- Conventional methods
- New methods
- Advantages and disadvantages
- How to approach health monitoring

The simplest solutions are sometimes the cleverest – but they are often wrong



http://readwrite.com/2011/10/17/

The Importance of Health Monitoring

Under existing conditions there are occasions when up to fifty per cent of animals sent to an institution never survive to the experimental stage. Moreover, even if they live to be used for research or assay, the death of their companions rightly casts suspicion on the survivors and the careful worker proceeds to use, as a precaution, twice as many animals as he would do if he had confidence in their health.

Conference on the Supply of Experimental Animals (1945)

(Kirk, R.G.W. (2010) *Isis* **101**(1): 62-94)

Why Health Monitoring?

- **Human health** – monitoring for the presence of *zoonoses*
- **Animal health** – Avoiding clinical disease, refining the experiment by reducing variables and so reducing animal numbers – 3Rs
- **Investigator's health** – avoiding unexpected results because of interference by infection

Health Monitoring *versus* Microbiological Monitoring

Health Monitoring *versus* Microbiological Monitoring

Infection is not synonymous with disease

The Aims of Microbiological Monitoring

- If an agent is present in the facility, to identify it in at least 1 animal or sample in the cohort tested
- To validate management practices in the facility
- Create an accurate picture of the microbiological status of a facility

Conventional Test Procedures

- **Bacteriology –** culture methods
- **Parasitology –** microscopy
- **Serological methods -** for viruses and some other agents

Potential Problems Associated with Bacteriology

- Contamination at necropsy of the tissues and organs to be sampled, thus obscuring infectious organisms.
- Loss of organisms due to overgrowth of other organisms eg *Proteus* spp
- Time consuming. Average time from sampling to final identification = 3-5 days
- Some organisms require special culture conditions
- Requires experienced personnel
- Sampling of sites of colonisation

Pitfalls of Parasitology

- Techniques may not be sensitive – wet preparations/flotation
- Sensitivity may be affected by parasite population dynamics
- Treatment will reduce sensitivity
- Identification is subjective – requires experience to identify organisms

SEROLOGY
Important Points to Consider

- Serology detects antibodies – the footprints of an infection
- It is an indicator of infection only
- Where possible, results should be supported by other data
- Cannot test immunodeficient animals – no antibodies

BEWARE FALSE POSITIVES/NEGATIVES

A short word on antibodies

http://www.medicine.mcgill.ca/physio/vlab/immun/backg.htm

- Diseased animals have not yet produced sufficient antibody to combat infection
- Animals submitted for diagnostic serology should not be submitted during this ‘lag’ phase. They will probably give a *negative* result.

Specimen Quality

- A valid test depends on specimen quality

- Results obtained from samples that are
  - haemolysed
  - lipaemic
  - contaminated

  are likely to be invalid

- Repeated freezing and thawing of serum will lead to diminished levels of antibody

PITFALLS OF HEALTH SURVEILLANCE– FALSE POSITIVES AND FALSE NEGATIVES

Reliability of the diagnostic test

Prevalence of the pathogen

Immune status

Genetic background

Sentinel or original strain

Type of housing

Sentinel Systems

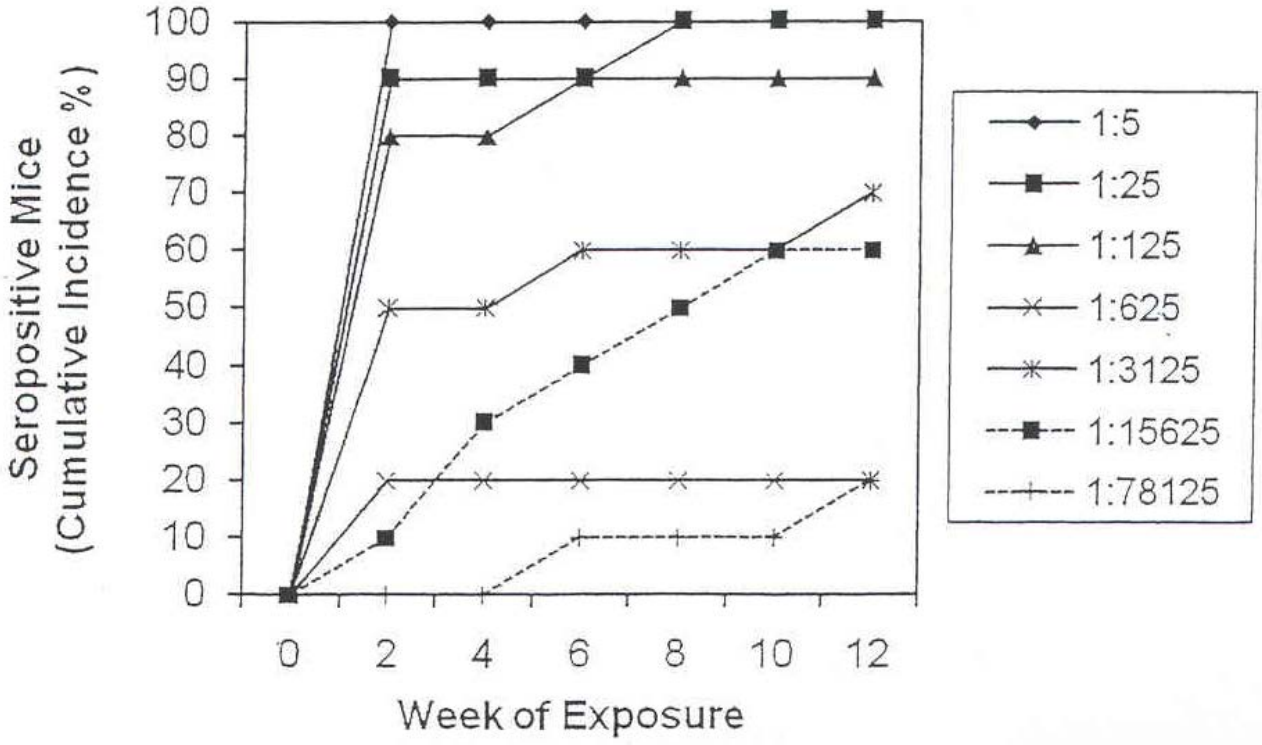
- **Soiled bedding** — not suitable to detect all organisms (Sendai, Pasteurella, some helicobacters)
- **In-contact sentinels** — efficient, but not always possible
- **Exhaust air** — not suitable to detect all organisms (MPV, rotavirus, helicobacter)
- **PCR on exhaust filters** — not suitable to detect all organisms

Ref: Compton *et al* (2004)

The sentinel programme should use a combination of systems – but this is difficult to achieve

Use of sentinels in health monitoring

Cumulative incidence of MPV rVP2
seropositive mice *vs* bedding dilution and exposure time



Besselsen (2008) *Comp Med* **58**, 140-144

Sample size (N)[b]	Assumed InfectionRate(%)											
	1	2	3	4	5	10	15	20	25	30	40	50
5	0.05	0.1	0.14	0.18	0.23	0.41	0.56	0.67	0.76	0.83	0.92	0.97
10	0.1	0.18	0.26	0.34	0.4	0.65	0.8	0.89	0.94	0.97	0.99	
15	0.14	0.26	0.37	0.46	0.54	0.79	0.91	0.95	0.99			
20	0.18	0.33	0.46	0.56	0.64	0.88	0.95	0.99				
25	0.22	0.4	0.53	0.64	0.72	0.93	0.98					
30	0.25	0.45	0.6	0.71	0.79	0.96	0.99					
35	0.3	0.51	0.66	0.76	0.83	0.97						
40	0.33	0.55	0.7	0.8	0.87	0.99						
45	0.36	0.69	0.75	0.84	0.9	0.99						
50	0.39	0.64	0.78	0.87	0.92	0.99						
60	0.45	0.7	0.84	0.91	0.95							
70	0.51	0.76	0.88	0.94	0.97							
80	0.55	0.8	0.91	0.96	0.98							
90	0.6	0.84	0.94	0.97	0.99							
100	0.63	0.87	0.95	0.98	0.99							
120	0.7	0.91	0.97	0.99								
140	0.76	0.94	0.99									
160	0.8	0.96	0.99									
180	0.84	0.97										
200	0.87	0.98										

[a] ILAR, 1976

Health monitoring and the 3Rs

Conventional methods require animals to be *sacrificed*

Conventional methods require animals to be *transported*

Conventional methods are designed for *'open cage'* facilities

Improvements in Housing – developments in HM

Image University College London

Image courtesy Harlan Laboratories

‘New’ Testing Procedures

- **PCR Testing – Multiplex PCR**
  - Reduces the need for prolonged sentinel screening for some agents
  - Samples may be pooled – bigger sample sizes and testing of individual cages
  - Relies on the principle of agents being shed – if an animal is not shedding, it does not pose a risk to the facility.
  - Enhanced sensitivity for some agents

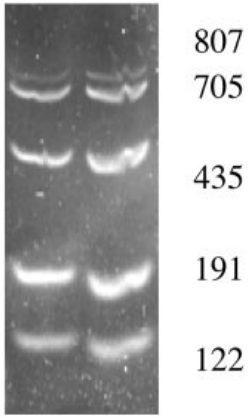


FIG. 2. Five-plex PCR for amplification of five targets in single reaction. Five sets of primers—*p17*, *p25*, *p30*, and 16S rRNA primers for *H. rodentium* (Hr1201f/1375r) and *H. typhlonius* (Hr163f/262r)—and five genomic DNAs each at concentrations of 5 ng (lane 1) and 0.5 ng (lane 2) plus 1 μl of fecal DNA were mixed in a single PCR.

Feng *et al*, 2005

Example – fur mites

- A persistent problem in mouse colonies (Arbona, Lipman *et al*, 2010)
- Soiled bedding sentinels are unreliable in detecting fur mites (Lindstrom *et al,* 2011)
- Ectoparasites are probably under-reported (test procedures lack sensitivity)
- PCR uses swab of cages or rodent pelts
- Avoids sacrificing animals
- Enhances quarantine

Images - RADIL

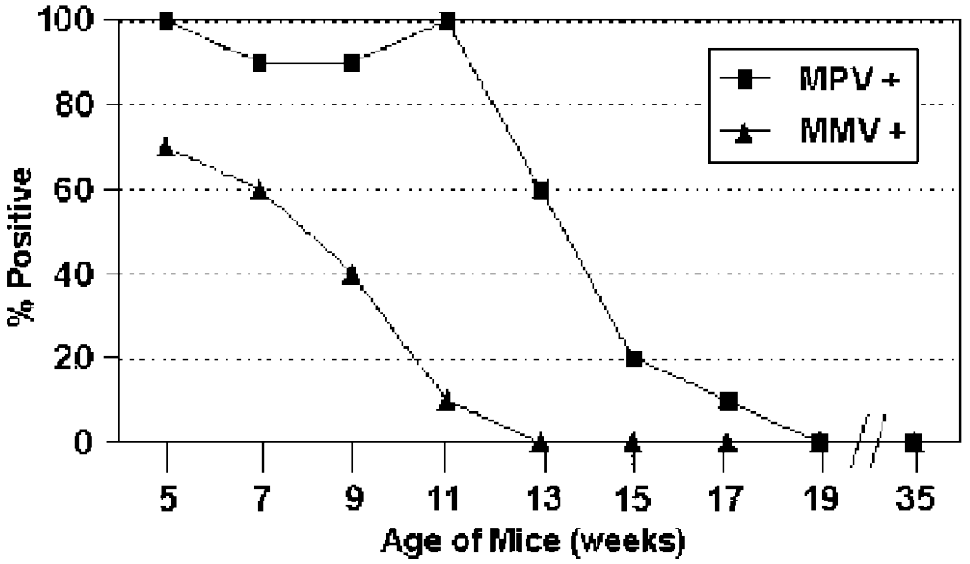
Mouse Parvovirus

Table 2 PCR and serology results

Parvovirus	Test material (assay)	No. positive/No. tested	% positive
MPV	Mesenteric lymph node (PCR)	35/35	100
	Faeces (PCR)	26/35	74
	Serum (ELISA)	35/35	100
MMV	Mesenteric lymph node (PCR)	22/35	63
	Faeces (PCR)	12/35	34
	Serum (ELISA)	23/35	66

MPV=mouse parvovirus, MMV=mice minute virus, PCR=polymerase chain reaction, ELISA=enzyme linked immunosorbent assay

Mouse faeces positive by PCR (10 SENCAR mice)



Bauer and Riley (2005). *Lab Anim* **40**, 144-152

Helicobacter

- Moerth, Mahabir *et al* (2008) found strikingly inconsistent PCR results between 9 laboratories
- Poynter *et al* (2009) – false positives and false negatives depending on primers used.
- Serology, culture and histopathology - unreliable

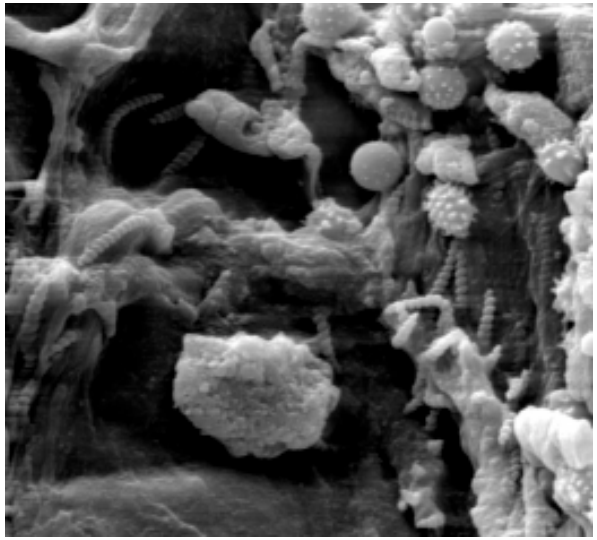


Photo: Idexx-RADIL

New options for serological testing

- Collection of dried whole blood spots onto filter cards (dried blood spot technology)
- No need to sacrifice the animal
- No need for centrifugation and potentially solves issues of haemolysis of samples
- 3-5mm DBS = 1-4ul serum
- No need to freeze or ship on dry ice

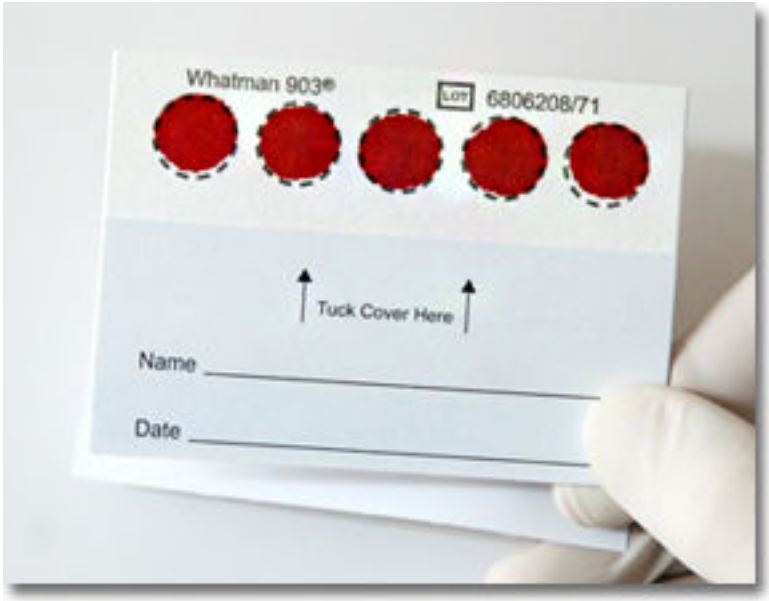


Image: BioMerieux-Diagnostics

Pathogens, Commensals and Opportunists

- **Pathogen** — **An organism that causes disease**
- **Opportunist** — **A usually harmless organism that can cause disease under favourable conditions**

- **Commensal** — **An organism that lives in or on another, deriving benefit, but not causing harm**

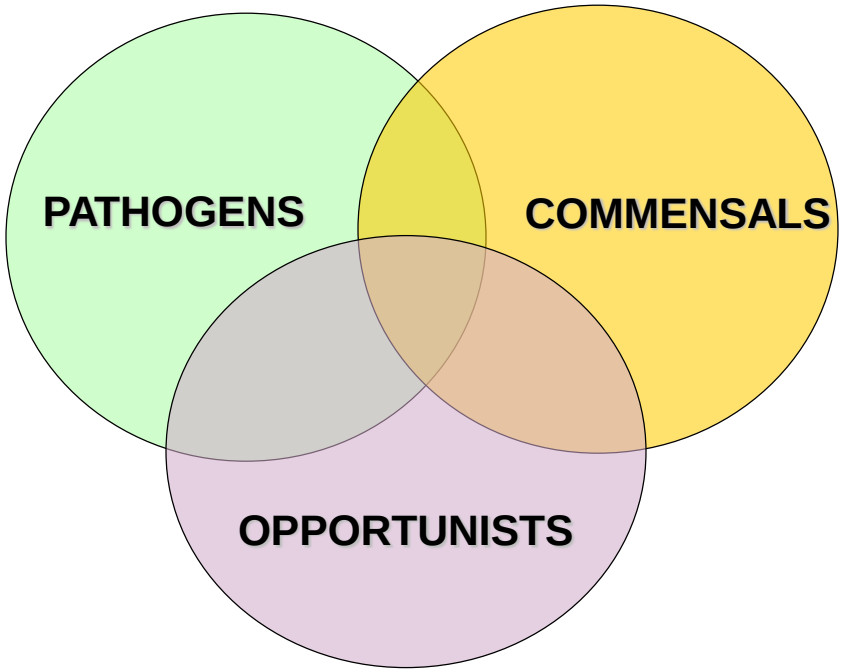
BUT…

Pathogens, Commensals and Opportunists

- **Pathogen** — **An organism that causes disease**
- **Opportunist** — **A usually harmless organism that can cause disease under favourable conditions**

- **Commensal** — **An organism that lives in or on another, deriving benefit, but not causing harm**

BUT…

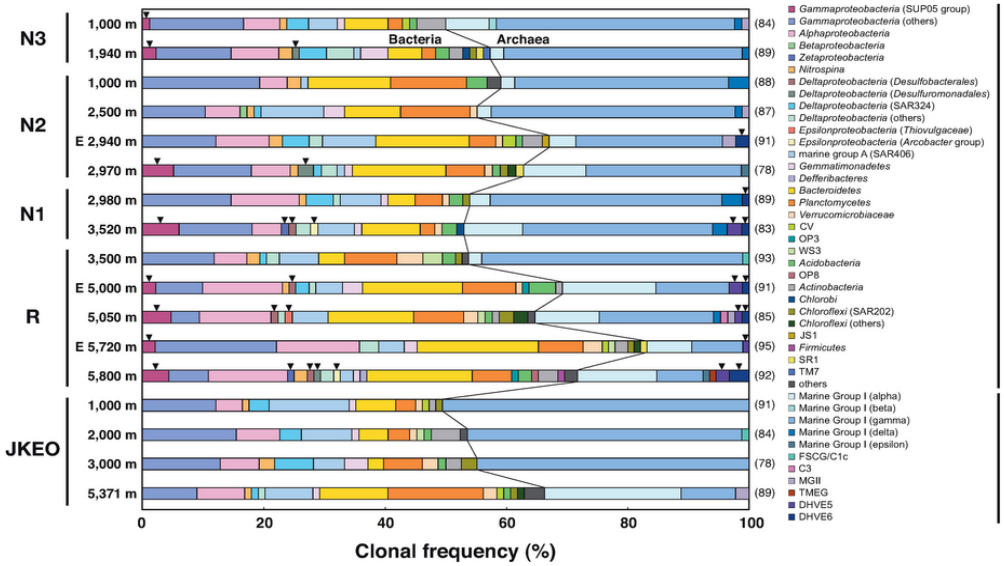


Increasing interest in the gut ‘microbiome’

- Ivanov *et* al (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria.
- Cani *et al* (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2 driven improvement of gut permeability.
- Hufeldt *et al* (2010). Variation in gut microbiota of laboratory mice is related to both genetic and environmental factors.
- Van de Merwe *et al* (1983). The resident faecal flora is determined by genetic characteristics of the host. Implications for Crohn’s disease.
- Gonzalo *et al* (2010). Spontaneous *Staphylococcus xylosus* infection in mice deficient in NADPH oxidase in comparison with other mouse strains.
- Pedrosa E *et al* (2011). Bacteria and spontaneous experimental colitis: immunological changes
- Imai A (1984). Endogenous infection in mice with streptozotocin-induced diabetes. A feature of bacterial translocation.

16srRNA Profiling

- **Microbiome** – the new genome
- 16s rRNA is highly conserved in bacteria



Nelson *et al* PLOS ONE DOI:10.1371/journal.pone.0094249

- Hypervariable regions can provide species-specific signatures sequences for bacterial identification
- Profiling used to identify and enumerate the organisms in a given sample
- Increased emphasis on the microbiome – the 10^{14} organisms residing in the gut and elsewhere
- Not used routinely

Advantages of new testing procedures

- Provide a greater sample size when testing IVCs
- Indicate agents that are being ‘shed’
- Potentially very sensitive
- Immunodeficient animals can be tested for viruses by PCR
- Potentially accelerate the process of quarantine
- 3Rs: reduce the numbers of animals sacrificed and transported

Disadvantages of new test procedures

- They have a limited track-record
- They rely on agents being ‘shed’ – intermittent shedding?
- They may be prone to false positive and false negative results
- Labour intensive for technicians

Summary

- Understand the aims of the health monitoring programme
- Sentinel programmes have significant drawbacks
- Conventional test methods are still valuable - but new methods have several advantages – eg 3Rs

The best successes come from combining the old with the new