Educational Workshop

EW05: Diagnosis of parasitic infections

arranged with
EFWISG (ESCMID Food- and Water-borne Infections Study Group) and ESGCP (ESCMID Study Group for Clinical Parasitology)

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Laetitia Kortbeek, Amsterdam, NL
Molecular diagnostics of intestinal parasites in epidemiology and patient care

Jaco J. Verweij

History

- Started in 1995 with differentiation of microscopically identical Entamoeba histolytica and Entamoeba dispar
- PCR used for differentiation and confirmation
- Further improvement of DNA isolation methods
- Introduction of real-time PCR provides
  - High sensitivity
  - High specificity
  - Multiplex several targets
  - (semi) quantitative results
  - Reduced contamination risk
- Multiplex detection of intestinal parasites in patientcare and epidemiology

Schistosoma multiplex real-time PCR

Entamoeba histolytica

- Amoebae
- Invasive disease
  - amoebic colitis
  - amoebic dysentery
  - amoebic liver abscess
- 100,000 deaths annually
- The cyst is the infectious form and is very resistant

Laboratory diagnosis of E. histolytica infections

- Microscopy
  - trophozoites in fresh or preserved stool samples (1876)
  - cysts in iodine stained formalin-ether concentrate
    - not very sensitive
    - 3 samples required
    - Differentiation from E. dispar not possible
- Antigen detection
  - detection of antigen in fresh stool samples
    - more sensitive
    - specific
- PCR
  - detection of DNA in fresh or ethanol preserved stool samples
    - even more sensitive
    - specific

Multiplex detection of diarrhoea causing protozoa in faeces using real-time PCR

Giardia lamblia

Cryptosporidium
Giardia lamblia

- Flagellate
- One of the main non-viral causes of diarrhoea
- Trophozoites attach to intestinal epithelial cells (non-invasive)
- The cyst is the infectious form and is very resistant

Laboratory diagnosis of Giardia infections

- Microscopy
  - trophozoites in fresh or preserved stool samples (1681)
  - cysts in iodine stained formalin-ether concentrate
  - not very sensitive
  - 3 samples required

- Antigen detection
  - detection of antigen in fresh or preserved stool samples
  - more sensitive
  - 2 samples required

- PCR
  - detection of DNA in fresh or ethanol preserved stool samples
  - more sensitive

KML Giardia

Validation Giardia in HGC-PCR

- 362 samples microscopy, antigen test and PCR.

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>Antigen test</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>-</td>
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<tr>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

- *10 samples of 4 patients in which Giardia infection is confirmed by microscopy and/or antigen test in a subsequent sample.
- 11 samples of 2 x 3 patients from 2 families in Giardia infection was confirmed in a family member.
- 3 samples of 3 patients in which only was positive 1 of 3 samples.

Cryptosporidium parvum/hominis

- Coccidia
- first human case in 1976
- severe diarrhoea in AIDS patients

A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply


We estimate that 403,000 people had watery diarrhoea attributable to this outbreak.
Laboratory diagnosis of Cryptosporidium infections

- **Microscopy**
  - Oocysts in fresh or preserved stool samples
  - Modified Ziehl-Neelsen
  - Auramine
  - Safranine
  - Monoclonal antibodies
  - Not very sensitive
  - Specificity and sensitivity of the Mab?

- **Antigen detection**
  - Detection of antigen in fresh or preserved stool samples
  - More sensitive?

- **PCR**
  - Detection of DNA in fresh or ethanol preserved stool samples
  - More sensitive

Modified Ziehl-Neelsen, Morgan et al 1998

Microscopy and real-time PCR for the detection of a Cryptosporidium infection in an immuno-compromised child with complaints of diarrhoea

<table>
<thead>
<tr>
<th>Date</th>
<th>Microscopy (ZN)</th>
<th>PCR</th>
<th>Ct value</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-05-2002</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-09-2002</td>
<td>+</td>
<td>+</td>
<td>32.7</td>
</tr>
<tr>
<td>17-09-2002</td>
<td>-</td>
<td>+</td>
<td>28.0</td>
</tr>
<tr>
<td>10-02-2003</td>
<td>+</td>
<td>+</td>
<td>26.8</td>
</tr>
<tr>
<td>18-02-2003</td>
<td>+</td>
<td>+</td>
<td>27.9</td>
</tr>
<tr>
<td>10-04-2003</td>
<td>-</td>
<td>+</td>
<td>37.1</td>
</tr>
<tr>
<td>29-04-2003</td>
<td>+</td>
<td>+</td>
<td>31.2</td>
</tr>
<tr>
<td>13-05-2003</td>
<td>+</td>
<td>+</td>
<td>27.8</td>
</tr>
</tbody>
</table>

In summary

- Three diarrhoea causing protozoa
- "Microscopy" is the classic diagnostic method
- Separate alternative methods have been developed and have proved to be more sensitive and specific
- Disadvantage: separate techniques for optimal detection of the separate parasites

Performance of molecular diagnostic approach in different populations

- Groningen, patients attending their general practitioner
  - E. histolytica; G. lamblia; Cryptosporidium
- Zwolle, patients attending their general practitioner and peripheral hospital
  - E. histolytica; G. lamblia; Cryptosporidium
  - D. fragilis
- Antwerp, patients attending the Travel Medicine Clinic
  - E. histolytica; G. lamblia; Cryptosporidium
  - S. stercoralis
- Malawi, population in which HIV infection is highly endemic
  - E. histolytica; G. lamblia; Cryptosporidium
  - E. bieneusi; Encephalitozoon

Results Groningen, general practitioner setting N=950

Study groups

- Microscopy n=1636
- Bacteriology n=1184
- real time PCR n=950

E. histolytica 0 0
Giardia lamblia (n=720) 41 67
Giardia lamblia (n=230) not done 15
Cryptosporidium not done 45
Helminths 0 not done
Non-pathogenic protozoa 34 not done

Culture

- Campylobacter spc. 90
- Salmonella spc. 29
- Shigella sonnei 2

Jaco J. Verweij
Molecular diagnostics of intestinal parasites in epidemiology and patient care
Results Zwolle, general practitioner and peripheral hospital N=397  
(L. Bruijnesteijn et al. In prep)

- **D. fragilis**  
  - Since its discovery, the pathogenicity of this organism has remained controversial  
  - No cyst stage  
  - Preserved (SAF) faeces

Results Zwolle, general practitioner and peripheral hospital N=397  
(L. Bruijnesteijn et al. In prep)

<table>
<thead>
<tr>
<th>E. histolytica/E. dispar</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. histolytica</td>
<td>1</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>29</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>2</td>
</tr>
<tr>
<td>D. fragilis</td>
<td>66</td>
</tr>
<tr>
<td>Non-pathogenic protozoa</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>8</td>
</tr>
<tr>
<td>E. nana</td>
<td>10</td>
</tr>
<tr>
<td>B. hominis</td>
<td>111</td>
</tr>
</tbody>
</table>

Study on the potential pathogenicity of **D. fragilis**

- Epidemiology of **D. fragilis** in relation with other diarrhoea causing pathogens  
- Development of genotyping methods and molecular epidemiology of **D. fragilis** in symptomatic and asymptomatic cases.
Results Antwerp Travel Clinic
N=2717

<table>
<thead>
<tr>
<th>E. histolytica/E. dispar</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. histolytica</td>
<td>152</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>158</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>13</td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>4</td>
</tr>
</tbody>
</table>

Strongyloides stercoralis diagnostics

- MICROSCOPY
  - Detection of L1-larvae in stool concentrates
  - Detection of L1-larvae in Baermann sediments
  - Detection of L3 larvae in stool culture

Malawi, population in which HIV infection is highly endemic (N=182)

<table>
<thead>
<tr>
<th>Schistosoma mansoni</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hookworm</td>
<td></td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td></td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td></td>
</tr>
<tr>
<td>Taenia spec.</td>
<td></td>
</tr>
<tr>
<td>S. haematobium</td>
<td></td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
<td></td>
</tr>
<tr>
<td>Cyclospora</td>
<td></td>
</tr>
<tr>
<td>Isospora belli</td>
<td></td>
</tr>
<tr>
<td>Dientamoeba fragilis</td>
<td>76</td>
</tr>
</tbody>
</table>

Microsporidia

Detection of Microsporidian DNA with PCR in faeces (Katzwinkel et al, 1996)

- Semi-nested multiplex PCR
- ITS rRNA gene
- Specific for E. bieneusi
- Specific for other Microsporidia (ie E. intestinalis)
Available (multiplex) real-time PCRs

<table>
<thead>
<tr>
<th>Targets with internal control</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. histolytica; E. dispar</td>
<td>Published</td>
</tr>
<tr>
<td>E. histolytica, G. lamblia, Cryptosporidium</td>
<td>Published</td>
</tr>
<tr>
<td>E. bieneusi, Encephalitozoon</td>
<td>Published</td>
</tr>
<tr>
<td>D. fragilis</td>
<td>Published</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>Published</td>
</tr>
<tr>
<td>I. belli</td>
<td>In press</td>
</tr>
<tr>
<td>N. americanus, A. duodenalis O. M. mucron</td>
<td>Published</td>
</tr>
<tr>
<td>S. mansoni, S. meleagridis</td>
<td>Published</td>
</tr>
<tr>
<td>Schistosoma genus</td>
<td>Submitted</td>
</tr>
<tr>
<td>S. stercoralis</td>
<td>In preparation</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>In preparation</td>
</tr>
<tr>
<td>O. felineus</td>
<td>In preparation</td>
</tr>
<tr>
<td>Toxoplasma</td>
<td>In use</td>
</tr>
<tr>
<td>Reisomer species</td>
<td>In use</td>
</tr>
<tr>
<td>Plasmodium species</td>
<td>Published</td>
</tr>
</tbody>
</table>

Most (cost) effective way to use these assays

- Is a different approach needed from the approach that is used in choosing conventional diagnostics?
- Can we choose test based on complaints?
- Is it useful to detect every parasite in each population?

E. bieneusi and Encephalitozoon spp. real-time PCR results in fecal samples from different patient groups

<table>
<thead>
<tr>
<th>Underlying condition</th>
<th>Number</th>
<th>PCR result</th>
<th>Ct-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV infection</td>
<td>16</td>
<td>E. bieneusi</td>
<td>15.8-36.5</td>
</tr>
<tr>
<td>Kidney transplantation</td>
<td>6</td>
<td>E. bieneusi</td>
<td>18.9-24.8</td>
</tr>
<tr>
<td>Hypogammaglobulinemia</td>
<td>1</td>
<td>E. bieneusi</td>
<td>21.6</td>
</tr>
<tr>
<td>Adoption (1 &amp; 4 years old)</td>
<td>2</td>
<td>E. bieneusi</td>
<td>29.8; 34.8</td>
</tr>
<tr>
<td>Traveler</td>
<td>1</td>
<td>E. bieneusi</td>
<td>23.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>E. bieneusi</td>
<td>17.9-21.6</td>
</tr>
<tr>
<td>HIV infection</td>
<td>2</td>
<td>Encephalitozoon spp.</td>
<td>22.4; 24.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>Encephalitozoon spp.</td>
<td>34.0</td>
</tr>
</tbody>
</table>

Patients from 1996-2005 N=33

E. bieneusi PCR in hospital population Ethiopia

<table>
<thead>
<tr>
<th>Underlying condition</th>
<th>Number</th>
<th>PCR result</th>
<th>Ct-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney transplantation</td>
<td>1</td>
<td>E. bieneusi</td>
<td>20.0</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td>1</td>
<td>E. bieneusi</td>
<td>39.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>E. bieneusi</td>
<td>21.6</td>
</tr>
<tr>
<td>Colitis ulcerosa</td>
<td>20-06</td>
<td>E. bieneusi</td>
<td>21.9</td>
</tr>
<tr>
<td>09-10-2006</td>
<td>E. bieneusi</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>18-10-2006</td>
<td>E. bieneusi</td>
<td>38.1</td>
<td></td>
</tr>
<tr>
<td>12-01-2007</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection</td>
<td>1</td>
<td>Encephalitozoon spp.</td>
<td>31.0</td>
</tr>
<tr>
<td>Kidney transplantation</td>
<td>1</td>
<td>Encephalitozoon spp.</td>
<td>32.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>Encephalitozoon spp.</td>
<td>38.9</td>
</tr>
</tbody>
</table>
Molecular diagnostics of intestinal parasites in epidemiology and patient care

Encephalitozoon in hospital population Ethiopia

E. bieneusi PCR in hospital population LUMC

Encephalitozoon in hospital population LUMC

E. bieneusi in hospitalized diarrhea patients - Malawi

Polymorphic sites in the ITS sequence

Van Lieshout et al. unpublished data

Jaco J. Verweij

Encephalitozoon in hospital population Ethiopia

E. bieneusi PCR in hospital population LUMC

Encephalitozoon in hospital population LUMC

E. bieneusi in hospitalized diarrhea patients - Malawi

Polymorphic sites in the ITS sequence

Van Lieshout et al. unpublished data
Phylogenetic tree based on *E. bieneusi* ITS sequences

Genotypes found in specific patient groups

1. Malawi Children HIV+  
   N=9  
   D, K, SP2, SP5

2. Malawi Children HIV-  
   N=4  
   K, SP1, SP2, SP6

3. Malawi Adults HIV+  
   N=21  
   K, PE5624, UG2145, SP2, SP3, SP4, SP6, SP7,

4. LUMC Adults HIV+  
   N=7  
   B(4x), D, K, SP8

5. LUMC Adults transplant pat.  
   N=5  
   C

6. LUMC Adults HIV-?  
   N=7  
   A, K, SP6, SP10
Cyclospora: An Emerging Foodborne pathogen

Pablo C. Okhuysen, MD
The University of Texas Health Science Center – Medical School at Houston, Texas, USA

Outline
- Characteristics of Cyclospora that distinguish it from other Apicomplexan parasites
- Life cycle and sporulation requirements
- Epidemiology and outbreaks
- Clinical Manifestations
- Diagnosis and Treatment

Cyclospora
- First human cases described in 1977
- Previously referred to as cyanobacterium-like bodies, fungal spores, blue-green algae, “large” cryptosporidia
- Confirmed as belonging to the Cyclospora genus in 1993
- Closely related to Eimeria, distantly related to Isospora, Sarcocystis and Toxoplasma
Pablo Okhuysen
Cyclospora - an emerging food-borne parasite

Host Specificity
- Cyclospora-like organisms have been identified in mice, rats, chicken, ducks, dogs, primates and others
- Coprophagy vs. zoonosis with Cyclospora other than C. cayetanensis
- Unknown if animals can serve as a reservoir for C. cayetanensis
- Attempts to infect animals have been unsuccessful except for the albino mouse
- ID50 for humans unknown, challenge studies with 200 - 49,000 oocysts unsuccessful

Cyclospora in Primates
- Study of 511 stool specimens in 11 distinct primates in 10 locations in Kenya
- Oocysts typed for 18S rRNA analysis:
  - Vervet monkeys (43/102, 42%)
  - Yellow and olive baboons (19/206, 9%)
  - Black and white colobus monkeys (19/76, 25%)
- No seasonality, high host specificity in primates

Characteristics
- Oocysts measure 8-10 um in diameter
- Contain two sporocysts, each one with four sporozoites
- Unsporulated phase: outer membrane, outer fibrillar coat, cell wall, and membrane
- Sporulated sporozoites show a membrane bound nucleus and micronemes

http://www.cdc.gov/ncidod/dpd
Pablo Okhuysen
Cyclospora - an emerging food-borne parasite

**Life Cycle**
- Sporulation
  - Outside the host
  - Optimal at 27 to 32 °C for 8 to 11 days
- Oocysts survive:
  - Freezing, 2% formalin
  - 2% potassium dichromate and chlorination

**FoodNet Active Surveillance 2006**

**Effects of Gamma Irradiation**
- At doses of 0.4 kGy excystation occurs but not infectious to mice
**Epidemiology**

- Occurs worldwide in clusters and sporadically
  - High prevalence in Nepal, Peru, Egypt, Haiti
  - An infrequent cause of travelers' diarrhea
  - Increased frequency in HIV co-infection and other immunosuppressive conditions
- Outbreaks have been related to:
  - Contaminated water storage tanks
  - Imported raspberries, mixed vegetables, basil

**Cryptosporidium vs. Cyclospora**

**Guatemalan Children**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cyclo</th>
<th>Crypto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Episodes / year (all)</td>
<td>0.20</td>
<td>0.22</td>
</tr>
<tr>
<td>Incidence</td>
<td>Peaks 1 yr</td>
<td>Constant</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.21</td>
</tr>
<tr>
<td>Isolation and Diarrhea</td>
<td>Declines with time</td>
<td>Remains constant</td>
</tr>
</tbody>
</table>

**Association**

- House Animals
- No latrine

*Bern et. al. Emerg Infect Dis 1999;5:766-74*
Molecular Epidemiology

- Small subunit rRNA allows for genotyping
- Intervening transcribed spacer (ITS) sequencing shows a high variability
  - Useful for linking cases to a single source
  - Parasites from patients can contain multiple ITS1 genotypes
    - Variability within the genome of a single clone
    - Multiple clones from a single source “clonal polymaritism”

Oliver et al. Int J Parasitol, 2001;31:1475-78

Clinical Manifestations

- In indigenous populations
  - Asymptomatic infection is common even in some immunocompromised patients
- Incubation period
  - One to 11 days
- Symptoms
  - Flu-like illness may precede diarrhea
  - Watery diarrhea (average of 6 stools/day)
  - Upper GI symptoms common, nausea, abdominal pain
  - Fatigue, myalgia, cramps, flatus,
  - Fever in 25% of cases

- Duration of illness 2 - 7 weeks
- Cyclic or relapsing
- Dehydration, weight loss
- Clinically cannot be differentiated from other causes of diarrhea such as Cryptosporidium, Giardia
- Case reports of reactive arthritis, Guillain Barre
Pablo Okhuysen
Cyclospora - an emerging food-borne parasite

Clinical Symptoms Outbreak
Wedding Reception 1998

- Diarrhea: 57 (100)
- Weight loss: 53 (93)
- Fatigue: 52 (91)
- Anorexia: 51 (90)
- Weakness: 46 (81)
- Abd. Pain: 43 (75)
- Duration >3 wk (60%)

Diarrhea: 57 (100)
Bloating: 36 (63)
Myalgia: 36 (63)
Chills: 29 (51)
Sub. Fever: 22 (39)
Vomiting: 21 (37)
Headache: 21 (37)
Bloating: 36 (63)

Myalgia: 36 (63)
Chills: 29 (51)
Sub. Fever: 22 (39)
Vomiting: 21 (37)
Headache: 21 (37)

Fleming, C. Arch Intern Med. 1998;158:1121-1125

HIV related

- Longer duration of illness
- CD4 <200 cells/mL
- Can involve the biliary tree and cause cholecystitis
- Responds to therapy but relapses are common
- Secondary prophylaxis indicated in HIV


Histopathology

- Can be detected in jejunal aspirates or in biopsy specimens
- In tissues, *Cyclospora* is identified in supranuclear cytoplasmic space
- Inflammation of submucosa
- Deposition of electron dense myelin-like deposits

### Diagnosis
- Microscopic exam
  - Twice the size of cryptosporidia (8-10 um)
  - Visualized in fresh mount
  - Acid fast staining with Ziehl-Neelsen or Kinyoun
  - Safranin and lacto phenol cotton blue stain oocysts uniformly
- Shedding mirrors clinical illness
- In prolonged cases, xylose absorption can be abnormal

http://www.dpd.cdc.gov

### Autofluorescence
- *Cyclospora* oocysts exhibit intense blue color when excited with UV light using the filter set at 330-365 nm
- Less intense green fluorescence with blue excitation filter at 450-490 nm
- Can be done on a wet mount without dyes and age does not affect this property

http://www.dpd.cdc.gov

### Treatment
- Trimethroprim / sulfamethoxasole
  - Two prospective, double blind, placebo controlled studies have been done using 160/800 mg
  - Recommended dose is twice daily for 7 days
  - In HIV infection, tmp/smx used q.i.d and then chronic suppression three times a week
  - Case reports of failures when using trimethroprim alone

Hogue CW Lancet. 1995;345:691-3
Treatment

- **Ciprofloxacin**
  - In a study in Haiti, found to be similar to tmp/smx in efficacy
  - Treatment failures reported

- **Nitazoxanide**
  - Case reports of efficacy
  - An alternative in sulfa allergic patients or failures with other antibiotics

Pierre Marty
Cutaneous leishmaniasis

Rapid Diagnosis of Cutaneous Leishmaniasis

Geographical distribution

Visceral Leishmaniases
Clinical presentation
Pierre Marty
Cutaneous leishmaniasis

Amastigotes (May Grünwald Giemsa stain)

M.E. Bougnoux
Cutaneous leishmaniases

The difficulty
Great variety of clinical presentations
Great polymorphism related to species and hosts!

Leishmania infantum
Campania, Italy

Leishmania major
Kairouan, Tunisia

Leishmania braziliensis
Bolivia & Brazil

Cutaneous leishmaniases

The difficulty
Similar clinical spectrum to leishmaniasis

- leprosy
- skin cancers
- mycobacteriosis
- cutaneous mycoses
- sarcoidosis
- insect bite granuloma … etc

common in leishmaniasis-endemic areas
Pierre Marty
Cutaneous leishmaniasis

Cutaneous Leishmaniases

Leishmania infantum
Nice area, France

Leishmania major
Biskra area, Algeria

Leishmania aethiopica
Ethiopia
Pierre Marty
Cutaneous leishmaniasis

Cutaneous Leishmaniases

Leishmania mexicana
Mexico

Leishmania infantum
Nice area
France

Leishmania killicki
Tunisia
Cutaneous Leishmania

Skin biopsy

or

Skin aspiration

Microscopic examination

- Histopathological study (fixed lesion biopsy)
- May Grünwald Giemsa smears stained (not fixed biopsy or aspiration)

Culture on Nicolle-Novy-McNeal or Schneider medium

(zymodeme identification)

Cutaneous biopsy: numerous amastigote forms

MGG x 400
Pierre Marty
Cutaneous leishmaniasis

Cutaneous biopsy: numerous amastigote forms
MGG x 1000

Cutaneous biopsy: numerous promastigote forms
culture

Modern diagnosis methods of Cutaneous Leishmaniasis

Molecular diagnosis
PCR on skin biopsy or aspiration
- fresh
- paraffin-embedded
more sensitive
more rapid identification but expensive
Serodiagnosis
western blotting
rapid tests
Molecular diagnosis of Cutaneous Leishmaniasis

- PCR based methods
- Particularly useful in cases with low parasite load
- Specificity 100%
- Sensitivity improved by 20-70% compared with conventional parasitological diagnosis (time consuming, clinical expertise)


Western blot in cutaneous leishmaniasis

WB profiles of some patients in different groups:

A: with active lesion(s)
B: with cured lesion(s)
C: without history of leishmaniosis
T: visceral leishmaniasis (L. infantum)

Serological rapid diagnosis tests in cutaneous leishmaniasis

Pierre Marty
Cutaneous leishmaniasis

**DiaMed-IT LEISH**

Dipstick using K39 recombinant antigen, an individual test with all required accessories packed in an aluminium foil and which could be defined as a bedside test.

**ID-PaGIA Leishmaniasis**

A Particle Gel ImmunoAssay based on an agglutination principle using particles coated with K39rAg. Card with 6 individual microtubes containing gel, useful to test large series.

**Global Results**

<table>
<thead>
<tr>
<th></th>
<th>Positive IT-Leish</th>
<th>Negative IT-Leish</th>
<th>Positive ID-PaGIA</th>
<th>Negative ID-PaGIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leishmaniasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL HIV- n = 46</td>
<td>98%</td>
<td>2%</td>
<td>98%</td>
<td>2%</td>
</tr>
<tr>
<td>VL HIV+ n = 30</td>
<td>53%</td>
<td>47%</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td>CL n = 39</td>
<td>49%</td>
<td>51%</td>
<td>56%</td>
<td>44%</td>
</tr>
<tr>
<td>Patent Leishmaniasis</td>
<td>5%</td>
<td>95%</td>
<td>11%</td>
<td>89%</td>
</tr>
<tr>
<td>Without Disease</td>
<td>0%</td>
<td>100%</td>
<td>1%</td>
<td>99%</td>
</tr>
</tbody>
</table>
Pierre Marty
Cutaneous leishmaniasis

Serological rapid diagnosis tests in cutaneous leishmaniasis (CL)

- 50% of the sera of CL were positive in both tests

For the diagnosis of CL, we underline the usefulness of the predictive value of a negative result

Conclusion

Classical diagnosis:
sensitivity related to microscopic experience
but time-consuming

PCR diagnosis:
great threshold sensitivity, necessity of a specialized lab
but expensive

Rapid serological tests:
great negative predictive value, easy to perform, cheap
but moderate sensitivity
How far have multiplex-PCR and quality assessment of PCR come?

H. Pelloux, Parasitology-Mycology
Teaching hospital, Grenoble, France and UMR 5163 UJF-CNRS.
On behalf of the ESGCP

Introduction
- Large topic
- Major improvements in the diagnosis of infectious diseases, particularly Parasitology, in the last decades
- Quality assessment is based on both quality controls (QC) and standard quality management procedures (ISO…)
- Recent developments of PCR (qualitative, quantitative, multiplex…) have modified the diagnosis of many parasitic diseases

Introduction
- QC for PCR are added to QC for other techniques used for the diagnosis of parasitic diseases which still remain useful
- Necessary in human medicine but also in veterinary medicine, study of the environment…
- Multiplex PCRs are the most sophisticated PCRs, applied to various pathogens
Herve Pelloux
How far have multiplex-PCR and quality assessment of PCR come?

Introduction

- References in PubMed:
  « Quality control Parasitology »: > 130 refs since the 90’s
  (much less specifically on QC for PCR)
  « Multiplex PCR Parasitology »: > 100 refs since the 90’s

- Impossible to analyse all of them
- Focus mainly on one parasitic disease as an example: Toxoplasmosis

QC PCR Toxo

- Only few papers in the literature focusing on QC for PCR for Toxoplasma

Guy et al, 1996
Pelloux et al, 1998
Costa et al, 2001
Bastien et al, 2007
Kaiser et al, 2007

These papers describe different QC that have been built for PCR Toxo

QC PCR Toxo


- Five European centers
- Amniotic fluid spiked with Toxo DNA
- Both false negative and false positive results
- Heterogeneity of techniques
- Need for external assurance scheme
Herve Pelloux

How far have multiplex-PCR and quality assessment of PCR come?

QC PCR Toxo

Table 1. Summary of polymerase chain reaction (PCR) protocols in five European laboratories.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Sample preparation</th>
<th>Target gene</th>
<th>PCR protocol</th>
<th>Unknown</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>genotype/PCR</td>
<td>T. gondii</td>
<td>single primer</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>B</td>
<td>genotype/PCR</td>
<td>T. gondii</td>
<td>single primer</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>C</td>
<td>genotype/PCR</td>
<td>T. gondii</td>
<td>single primer</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
<td>genotype/PCR</td>
<td>T. gondii</td>
<td>single primer</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>E</td>
<td>genotype/PCR</td>
<td>T. gondii</td>
<td>single primer</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

QC PCR Toxo

Table 2. Summary of results for the detection of Toxoplasma gondii DNA in amniotic fluid using the polymerase chain reaction.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>No. of positive cultures in 0.5 ml</th>
<th>No. of negative cultures</th>
<th>Total no. positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>


- Collaborative European study
- 15 centers
- 12 aliquots of amniotic fluid spiked with tachyzoites of the RH strain of Toxoplasma
- Heterogeneous PCRs and results
- Need for an external quality assurance scheme
Herve Pelloux
How far have multiplex-PCR and quality assessment of PCR come?

QC PCR Toxo

Costa J.M., et al, Bone Marrow Transplant. 2001

-4 European laboratories
-Tachyzoites of the S3 strain of Toxoplasma diluted in EDTA blood
-Diversity of the techniques
-Diversity of the results
-Need for quality controls with reference samples
How far have multiplex-PCR and quality assessment of PCR come?

**Table 1. Overview of the methods and primers used in the French external quality assessment scheme for molecular detection of *Toxoplasma gondii* in 2004**

<table>
<thead>
<tr>
<th>Type of method</th>
<th>DNA method</th>
<th>PCR-based method</th>
<th>DNA target</th>
<th>Primer used</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA extraction method</td>
<td>Commercial kit</td>
<td>B1 gene, IP</td>
<td>RED-ROD (12) L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QIAamp DNA Blood Kit</td>
<td>B1 gene, IP</td>
<td>RED-ROD (12) L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QIAamp DNA Blood Kit</td>
<td>B1 gene, IP</td>
<td>RED-ROD (12) L.</td>
<td></td>
</tr>
</tbody>
</table>

*The number of DNA targets and primer pairs may differ from that of participants, as several laboratories used two different PCR methods.*

**QC PCR toxo**


- Analysis of the French QC for PCR Toxo 2002-2004
- QC still organised
- 23 laboratories in France
- Amniotic fluid containing different concentrations of *Toxoplasma*, naturally infected
- Results better than in the previous published studies
- Heterogeneity of techniques
- This external quality assurance scheme allows to improve laboratory practice and communication among labs
Herve Pelloux
How far have multiplex-PCR and quality assessment of PCR come?

**Table 2.** Overall results for the molecular detection of *Toxoplasma gondii* in the French external quality assessment in 2002–2004

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample*</th>
<th>n</th>
<th>False-positive*</th>
<th>False-negative*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>-</td>
<td>21</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2003</td>
<td>-</td>
<td>22</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(1x10^6)</td>
<td>22</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2004</td>
<td>-</td>
<td>33</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(1x10^5)</td>
<td>23</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(1x10^4)</td>
<td>23</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(1x10^3)</td>
<td>23</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>111</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

* n: negative sample; +: positive sample estimated parasite concentration in lymphocytes/ml, see text.


- Proficiency panel designed to assess the performance of techniques for the detection of *Toxoplasma* in amniotic fluid
- Lyophilised samples of dilution of *Toxoplasma* in amniotic fluids (RH strain)
- 33 labs in 17 countries
- Both false positive and false negative results
- Need for improvement of molecular detection of *Toxoplasma*

**Table 3.** Composition of the panel and number of correct results per sample and type of assay

<table>
<thead>
<tr>
<th>Target conc. (Tg/ml)</th>
<th>Number correct (% correct)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All tests</td>
</tr>
<tr>
<td></td>
<td>n=38</td>
</tr>
<tr>
<td>1000</td>
<td>28 (100)</td>
</tr>
<tr>
<td>100</td>
<td>28 (100)</td>
</tr>
<tr>
<td>20</td>
<td>29 (76.3)</td>
</tr>
<tr>
<td>10</td>
<td>24 (62.2)</td>
</tr>
<tr>
<td>5</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td>0</td>
<td>26 (60.2)</td>
</tr>
</tbody>
</table>

* 1x ABI 7500, 3x ABI PRISM 7700, 1x ABI PRISM 7900, 1x BioRad IQ Cycler.


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How far have multiplex-PCR and quality assessment of PCR come?

The range of nucleic acid extraction methods used in the Toxoplasma study

<table>
<thead>
<tr>
<th>Extraction methods</th>
<th>%</th>
<th>Mean performance score (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIAamp DNA Mini/blood-kit</td>
<td>11</td>
<td>10.4 ± 2.0</td>
</tr>
<tr>
<td>Roche MagnaPure</td>
<td>7</td>
<td>8.3 ± 1.8</td>
</tr>
<tr>
<td>Roche high pure viral NA kit</td>
<td>6</td>
<td>11.7 ± 0.8</td>
</tr>
<tr>
<td>Qiagen column (other)</td>
<td>5</td>
<td>8.0 ± 1.4</td>
</tr>
<tr>
<td>Extracellular (biolase)</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Boiling</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Other (not specified)</td>
<td>6</td>
<td>9.5 ± 2.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>38</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Conclusion:

- External quality control schemes now exist (national or international level)
- Numerous issues concerning the QC: Toxo strain, sample, DNA or parasite, storage, number of aliquots…
- Heterogeneity of PCRs (in house++)
- Need for a strong validation of each of them

- These validation schemes are even more important when the techniques used are more sophisticated
- For instance: PCRs with internal control are in fact a kind of multiplex PCR…
Herve Pelloux
How far have multiplex-PCR and quality assessment of PCR come?

**Multiplex PCR**
- Most sophisticated PCR (qualitative or quantitative)
- Appeared in the early 90's
- More than 100 papers for the keyword « multiplex PCR parasitology » in PubMed
- Main application: simultaneous detection of different strains or species or genders in one sample
- But not only…

**Multiplex PCR**
- Examples of co-detections that have been described
  - Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum
  - 4 species of Plasmodium
  - Neospora caninum, Toxoplasma gondii
  - Entamoeba histolytica, Entamoeba dispar

**Multiplex PCR**
- Chloroquine resistant or not strains of Plasmodium falciparum
- Strains or species of leishmania
- Enterocytozoon bieneusi, Encephalitozoon spp.
- Viruses, Toxoplasma gondii
- … and others
A large number of parasites concerned by multiplex PCR
Herve Pelloux
How far have multiplex-PCR and quality assessment of PCR come?

Multiplex PCR

One example: *Toxoplasma gondii*

Usefulness of multiplex PCR for *T. gondii* (chronological)
- Co-detection with EBV in CSF of AIDS patients
- Recognition of *T. gondii* stage-specific genes
- Co-detection with viruses in posterior uveitis
- Differentiate *Neospora caninum* and *T. gondii* in dog
- Typing strains of *T. gondii* (congenital, immunocompromised, environment)
- Co-detection with viruses in congenital infections and bacteria, viruses in granulomatous lymphadenitis

Multiplex PCR

Dabil *et al.*, 2001

Validation of a diagnostic multiplex PCR assay for infectious posterior uveitis

→ To validate multiplex PCR on vitreous biopsy in posterior uveitis
→ CMV, HSV, VZV, *Toxoplasma gondii*
→ Comparison multiplex/monoplex on 21 vitreous specimen

Multiplex PCR

Results:

- Monoplex: detection of fewer than 10 genomes of VZV and 100 genomes of HSV, CMV, T. gondii
- Multiplex: loss of sensitivity (< 1 log, monoplex positive 18/21 samples, multiplex: 15/18)
- No false positive

Conclusion:

- Multiplex avoids serial testing (expensive, time consuming, limited volume of sample)
- But: declining sensitivity and complexity of annealing reactions
How far have multiplex-PCR and quality assessment of PCR come?

**Multiplex PCR**

*Ajzenberg D. et al, 2005*

- Designed for multilocus strain typing of *Toxoplasma gondii*.
- Height polymorphism of 5 microsatellites markers.
- Multiplex PCR easy to use (1 PCR needed to perform multilocus typing with five markers), rapid (1 day), adapted to large series.

**Conclusion:**

- Multiplex PCR in Parasitology
  - Often « in house »
  - Not commercialised
  - Should be carefully validated (see QC…)
  - Saves time but in each all the targets are used even if not medically justified (« screening »)
  - Future?

**Perspectives:**

- In the field of Mycology and Bacteriology:
  - one multiplex PCR commercialised for severe sepsis
  - Cost/effectiveness under evaluation
How far have multiplex-PCR and quality assessment of PCR come?

Conclusion:

- QC and multiplex PCR are major improvements in the field of Parasitology in the last decade
- QCs are now available
- Multiplex PCRs are emerging.
  cost/effectiveness is a major issue
Rapid diagnosis of parasitic infections

**Intestinal parasites**

Titia Kortbeek

Centre for Infectious Disease Control Netherlands, Bilthoven

---

**Rapid:**

- **Is it necessary to have a quick answer?**
  - Depending on request of doctor or patient
    - For intestinal parasites: duration of symptoms usually long:
      - (The patient is not sitting in the waiting room.)

- **Efficiency laboratory:**
  - Staining methods are time consuming
  - Training technicians
  - Motivation (most samples are negative)
  - Level knowledge sender
Laetitia Kortbeek
Intestinal parasites

- Which techniques are available
  - Preference microbiologist
  - Available equipment

- Which parasites do you want to detect:
  - All
  - Only Giardia and Crypto

• Hospital setting or general practitioner
  - Academic - peripheral hospital

• Patients: YOPI
  - Very Young, very Old, Pregnant and Immunocompromised

• Other available information:
  - Season
  - Duration of symptoms

Intestinal parasites

- Valid request from clinicians?
  - Algorithm for diagnostic request in gastro-enteritis patients of general practitioners
  - Sometimes specific information important
    - E.g. contact with animals
  - Clinical symptoms (fever, blood with stool, mucus) cannot be used to exclude an diagnosis or select patients
### Intestinal parasites

Available rapid tests for intestinal parasites:

**Methods:**
- ELISA antigen detection
- Dipstick
- Cassette
- Direct fluorescence antibody assay

**Parasites: protozoa:**
- Cryptosporidium
- Giardia
- *E. histolytica/dispar*

### Age and Season

<table>
<thead>
<tr>
<th>Age</th>
<th>Season</th>
<th>Duration of Symptoms</th>
<th>Diagnostic Request</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 yr</td>
<td>Dec-May</td>
<td>1-3 days</td>
<td>Rota (52%); NLV (13%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-7 days</td>
<td>Rota (22%); Adeno (22%); NLV, SLV, Crypto (7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;7 days</td>
<td>NLV (15%); Rota, Adeno (7%)</td>
</tr>
<tr>
<td></td>
<td>June-Nov</td>
<td>1-3 days</td>
<td>Rota (27%); NLV (13%); Camp., Adeno (7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-7 days</td>
<td>Salm (17%); Camp (11%); Rota, Adeno, Crypto (4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;7 days</td>
<td>Giardia (9%); Crypto (6%); Adeno (6%)</td>
</tr>
<tr>
<td>&gt;5 yr</td>
<td>Dec-May</td>
<td>1-3 days</td>
<td>Camp (16%); NLV (24%); Rota (7%); Astro (6%); Salm (4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-7 days</td>
<td>Camp (18%); Rota, Crypto (5%); Astro, Salm, (4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;7 days</td>
<td>Camp, Giardia (4%)</td>
</tr>
<tr>
<td></td>
<td>June-Nov</td>
<td>1-3 days</td>
<td>Camp (21%); Salm (7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-7 days</td>
<td>Camp (20%); Giardia (5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;7 days</td>
<td>Giardia (10%); Camp (3%)</td>
</tr>
</tbody>
</table>
Intestinal parasites

Important criteria:
- Reliability
- Sensitivity and specificity
- Easy to use
- Costs
- Hands on time
- Literature comparing results in same setting
- NVP and PPV are depending of prevalence

What is the golden standard?
- Microscopy or PCR?
- Multiple sampling?
Giardia

Data Dr. Theo Mank, Haarlem, the Netherlands
Setting: diarrhea patients general practitioners, the Netherlands

Comparing
- Rida Quick
- ImmunocardStat
- X/Pect
- Triage

- Sensitivity: >93 to 95%
- Specificity: >98%
Laetitia Kortbeek
Intestinal parasites

Garcia et al 1996: evaluation 9 commercial kits
Setting: human feces in 10% formaline

- DFA Techlab
  - Giardia/Crypto IF; Crypto IF
- DFA Meridian Merifluor Crypto,
- EIA Alexon ProspeT
  - Giardia Crypto
- EIA Meridian
  - Premier Giardia; Premier Crypto
- Techlab CELISA
- EIA Trend Giardia
- EIA Cambridge Giardia

Reference test: DFA Meridian Merifluor Crypto/Giardia

Conclusions:
DFA Giardia and DFA Crypto:
- Sensitivity 100 %
- Specificity 100 %
EIA Giardia
- Sensitivity 94-99 %
- Specificity 96-100 %
EIA Crypto
- Sensitivity 94-99 %
- Specificity 96-100 %


Evaluation of the immunochromatographic CORIS Giardia-Strip test
Compared to a commercial ELISA-copraantigen test (ProspeT Giardia-ELISA microplate assay; Remel, Lenexa, KS, USA)

CORIS Giardia-Strip test
- Sensitivity 58%
- Specificity 99 %
Laetitia Kortbeek
Intestinal parasites

Comparing microscopy, real-time PCR and ImmunocardStat!

- Setting: gastroenteritis patients Groningen (Northern Netherlands)

Microscopy TFT:
- SAF preserved
- iodinestained, wet-mount preparation: suspect: chlorazol-black stain
- Unpreserved:
- Ridleyconcentration: iodinestained, wet-mount preparation

Microscopy,
Sensitivity 99%
Specificity 97%

real-time PCR
Sensitivity 100%
Specificity 92%

ImmunocardStat!
Sensitivity 98%
Specificity 100%


- Setting: outpatient clinic of the Institute of Tropical Medicine; diarrhoea and travelling

Sensitivity Giardia:
Ridascreen Giardia 82%
Rida Quick Giardia 80%
Rida Quick Combi 80%
Giardia-Strip 44%

Sensitivity Cryptosporidium:
Rida Quick Cryptosporidium 88%
Ridascreen Cryptosporidium 82%
Rida Quick Combi 82%
Cryptosporidium-Strip 75%

The specificity of all tests was ≥ 98%.

Weizel continued

- No cross-reactions with other intestinal parasites
- 68 (of 220 samples): other intestinal parasites

Conclusion:
- the copro-antigen assays: less time-consuming
- easier to perform
- but were less sensitive

"Thus, these tests might be a useful addition to, but not a substitute for microscopical methods in the diagnosis of travel-associated giardiasis and cryptosporidiosis."
Cryptosporidium

Magi Par. Res. 2005

Diagnostics Cryptosporidium:

- PCR, Kinyoun acid-fast stain, ImmunoCard STAT!

Setting: 127 diarrhoea immunocompetent patients in hospital Italy

<table>
<thead>
<tr>
<th>Patient Known Positive</th>
<th>ImmunoCard STAT! Cryptosporidium/Giardia Rapid Assay</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TG</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TI</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SG</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Significance of positive antigen test-negative microscopy and/or PCR?

- Recent recovery
- False positive

Conclusion:

For immunocompetent persons (and hence with few oocysts, if positive), microscopy test with Kinyoun stain currently seems to be the best approach in the hands of trained microscopists examining a large number of microscopic fields. Besides, this method is cheap even when used for a small number of samples.

Recall of ImmunoCard STAT

- Centers for Disease Control and Prevention (CDC).
- The Colorado Department of Public Health and Environment (CDPHE) has determined that a fourfold increase in the number of reported cryptosporidiosis cases in Colorado during January--February 2004 might be attributed primarily to false-positive test results. Since January 1, 2004, a total of 13 in-state cases and one out-of-state case were reported to CDPHE. During the previous 7 years, an average of three cases were reported during January--February. Of those 13 cases, rapid testing was performed by using the ImmunoCard STAT!® Cryptosporidium/Giardia Rapid Assay (Meridian Bioscience, Inc., Cincinnati, Ohio). This assay is a solid-phase qualitative immunochromatographic assay designated to detect and distinguish between Giardia intestinalis (lamblia) and Cryptosporidium parvum in aqueous extracts of human fecal specimens. Seven of these samples were tested by using lot no. 081093 (expires August 11, 2004). Of the seven samples that tested positive initially for Cryptosporidium with this lot number, four were retested by using other, more specific tests. One patient sample was positive by direct microscopy, one was negative by direct microscopy, and two were negative by direct fluorescent-antibody testing. The results of testing for Giardia intestinalis with these kits are unclear. Several other states have noted increases in the number of reported cryptosporidiosis cases that also might be associated with use of these rapid assays.

Manufacturer's Recall of Rapid Cartridge Assay Kits on the Basis of False Positive Cryptosporidium Antigen Tests --- Colorado, 2004

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- Meridian Biosciences, Inc., has voluntarily recalled two lots (lot no. 081077 [expires July 11, 2004] and lot no. 081093 [expires August 11, 2004]). CDC recommends reconfirmation of positive test results obtained with any ImmunoCard STAT!® Cryptosporidium/Giardia Rapid Assay tests from these lots. In addition, several states have determined that the ImmunoCard STAT!® Cryptosporidium/Giardia Rapid Assay test is no longer in widespread clinical use.

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Laetitia Kortbeek
Intestinal parasites


Setting: diagnostic laboratory in Germany; 738 stoolsamples

Microscopy:
- Concentration and iodine- wetmounted preparation
- Dried unstained slide: Crypto
- EZ4 Ridascreen Giardia and Ridascreen Cryptosporidium

Pre-screened positive specimens
- RIDAQuick Giardia,
- RIDAQuick Cryptosporidium
- RIDAQuick Cryptosporidium/Giardia Combi (R-Biopharm)

Conclusion: easy to use; rapid to perform; sensitive and specific


Diagnostic methods for differentiation of Entamoeba histolytica and Entamoeba dispar in carriers: Performance and clinical implications in a non-endemic setting

Unpreserved faecal samples
Microscopic examination; suspected to contain Entamoeba histolytica/Entamoeba dispar cysts or trophozoites

Reference test: real-time PCR.

416 patients: results real time PCR:

In 283 patients (68%) DNA of E. histolytica or E. dispar positive
- 6 patients with amebic colitis (2%)
- 19 carriers of E. histolytica (6.7%)
- 258 carriers of E. dispar (91.2%)

In 133 patients (31%) no DNA of E. histolytica or E. dispar could be amplified in the stool samples (controls)
Visser continued

Entamoeba test Techlab: monoclonal ab against *E. histolytica* and *E. dispar*

*E. histolytica* II Techlab: only monoclonal ab against *E. histolytica*

- Sensitivity: 59%
- Specificity: 98%

- Sensitivity: 71%
- Specificity: 100%

Serology: sensitivity: 81.3% and specificity: 95.2%.

Serology non endemic country: sensitivity 90% and specificity 98.1%.

In comparison to real-time PCR the performances of Entamoeba test™ and *E. histolytica* II™ lacked sensitivity for a reliable diagnosis of *E. histolytica*/*E. dispar* infection in a non-endemic setting.

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**E. histolytica/dispar in the Netherlands**

- In GE study GP patients (1996-1998):
  - 9/857 cases and 4/574 controls
- In population based study (1998):
  - 1/703 cases and 0/673 controls
- Recent study in Groningen: 930 cases GP:
  - no cases of Entamoeba

- *R. ten Hove et all (2007)* Detection of diarrhoea-causing protozoa in general practice patients. The Epidemiology of multiplex real-time PCR. Clinical Microbiology and Infection 13 (10), 1001–1007

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**Is it necessary to type Entamoeba histolytica/dispar?**

WHO guidelines


[Note: The WHO guidelines mention the necessity of typing *E. histolytica* and *E. dispar* based on certain criteria, including symptoms and clinical presentations.]

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**References**

- Entamoeba (R. ten Hove et al. 2007) Detection of diarrhoea-causing protozoa in general practice patients, The Epidemiology of multiplex real-time PCR. Clinical Microbiology and Infection 13 (10), 1001–1007
Conclusions

- Most rapid tests are easy to perform and fast
  - Sensitivity and specificity overall is OK
    - Be aware that sometimes batches can go wrong
  - Applicability depends on setting of the lab and type of patients
  - *E.histolytica / E.dispar* in non endemic patients: send these rare positive samples to specialized centers

- Importance of other parasites
  - Travel related
  - *Dientamoeba*

Thank you!!