A New Multiplex Real-time PCR Assay For Detection Of Intestinal Parasites

Dr. Andreas Simons
R-Biopharm Group

- Worldwide provider of diagnostic assay solutions
- Offers a variety of test kit methodologies
R-Biopharm Headquarters – Darmstadt, Germany

ISO 9001
ISO 13485

Worldwide ~ 500 employees
RIDA®QUICK rapid tests

RIDASCREEN® ELISA

RIDA®GENE, SureFood® and GEN-IAL® PCR
Worldwide Distribution

- R-Biopharm AG
- R-Biopharm Inc
- R-Biopharm España S.A.
- R-Biopharm Rhône Ltd
- R-Biopharm France
- R-Biopharm Italia Srl
- RefLab ApS
- R-Biopharm Analysis Systems Trading Co Ltd, China
- R-Biopharm Latinoamérica S.A.
- R-Biopharm Brasil Ltda
- R-Biopharm Australia Pty Ltd
Phoenix AirMid Biomedical

- Established in Canada since 1992
- Located in Oakville, ON
- Distributor and technical provider of medical diagnostic assays and instrumentation across Canada for over 15 international manufacturers
- Specializing in infectious & autoimmune diseases, cancer biomarkers, food intolerance and allergens, blood gases, and pathology diagnostics
- Offers a wide variety of assay methodologies (POC, IFA, RIA, WB, EIA, RT-PCR/multiplexing technology)
Guess the Parasite........
Giardia lamblia

E. histolytica

C. parvum

D. fragilis
Parasites – “The Uninvited Guests”

- Intestinal Parasites are classified into Protozoa and Helminths:
  
  **Protozoa**
  - Giardia
  - Cryptosporidium
  - Entamoeba histolytica/dispar
  - Dientamoeba fragilis
  - Cyclospora
  - Blastocystis hominis

  **Helminths**
  - Ascaris
  - Trichuris
  - Hookworm
  - Strongyloides
  - Schistosoma
  - Fasciola
  - Taenia

- Enteric protozoa affect millions of people each year worldwide
- Major etiological agents of parasitic diarrhea are: Giardia lamblia, Cryptosporidium spp., Dientamoeba fragilis and Entamoeba histolytica.
Giardia lamblia: Disease Burden

- Most frequent protozoal enteropathogen worldwide
- WHO estimates the prevalence of symptomatic giardiasis about 200 million cases worldwide
- Infects 2% of adults and 6-8% of children in developed countries worldwide.
- Nearly 33% of people in developing countries have had giardiasis.
- The CDC estimates about 77,000 cases of giardiasis each year in the United States (2010 ~ 20,000 cases reported)

3. Centers for Disease Control and Prevention. MMWR 2012;61(5)
Cryptosporidium parvum: Disease Burden

- **Prevalence**: 3% in industrialized countries (IN) 10% in developing countries (DC)

- **Cause of Diarrhea**:
  - Immunocompetent: 2% in IN, 6% in DC
  - Children: 7% in IN, 12% in DC
  - HIV: 14% (6 - 70%) in IN, 24% (9 - 48%) in DC

- CDC 2010 ~ 9,000 cases reported in the US

Entamoeba histolytica: Disease Burden

WHO estimates that about 50 million people worldwide suffer from amoebiasis each year, resulting in 100,000 deaths annually.

Dientamoeba fragilis: Disease Burden

- *D. fragilis* has a worldwide distribution that has been shown to cause acute gastrointestinal disease
- Prevalence varies from 0.3% to 52%

Detection methods for Intestinal Parasites

Conventional method
• Microscopy

New methods
• ELISA
• Rapid tests (lateral flow)
• Immunofluorescence
• Real-time PCR
### Microscopy – Conventional Method

**Advantages:**
- Simple equipment
- Cheap reagents
- Relatively quick results for a single sample
- Most intestinal parasites can be detected

**Disadvantages:**
- Trained personal needed
- Time consuming
- Low throughput per technologist
- Sample preparation
  - concentration method
  - Staining
- Sensitivity (Int. Protozoa ~ 60%)
- Specificity
  - lack of morphological discrimination
In conclusion, although common helminths were reliably diagnosed by European reference laboratories, there was only moderate agreement between centres for pathogenic intestinal protozoa.

Real-time PCR

- Rapid and Robust
- Sensitive
- Specific
- No cross-contamination
- Independent of specimen viability
- Easy Assay modification
- Controlled
- Multiplex testing
- Automatable
- Quantified outcome (Cp)
Method Comparison

**Entamoeba histolytica**

- **Microscopy**: 14%
- **Antigen ELISA**: 41%
- **PCR**: 98% (100% Sensitivity, 96% Specificity)

**Giardia lamblia**

- **Microscopy**: 63%
- **IFT**: 77%
- **PCR**: 97% (100% Sensitivity, 90% Specificity)

**C. parvum**

- **Microscopy**: 71%
- **IFT**: 80%
- **PCR**: 97% (100% Sensitivity, 98% Specificity)

*Tannich E, ECCMID 2012*
RIDA®GENE Parasitic Stool Panel

- Multiplex real-time PCR Assay
- Can be run on commonly used real-time PCR instruments
- Can be used with different extraction systems (manual/automatic)
- Time to result < 1.5 hours
- Internal Control
- Complete test kit (all reagents included)
- CE-marked and Health Canada approved (pending)
RIDAGENE Parasitic Stool Detection Format: Taqman/Hydrolysis Probes & Intercalating Dye

- TaqMan probe is labeled with a fluorescent reporter at the 5' end and a quencher at the 3' end

HRM dye (VIC Channel)
RIDA®GENE Parasitic Stool Panel

Matrix: Stool
Targets: Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum, Dientamoeba fragilis (18s-ITS)
Sensitivity: \( \leq 5 \) DNA copies/rxn
Reactions: 100
Time to result: 60 min (Tm: +20 min)
Cycler: LightCycler® 480II, SmartCycler®, ABI 7500, m2000rt, Mx series, Rotor-Gene Q
Results of RIDA®GENE Parasitic Stool – LC480

Giardia lamblia (465/510)

E. histolytica (533/610)

C. parvum (618/660)

IAC (533/580)
Results of RIDA®GENE Parasitic Stool – LC480

D. fragilis
(Melting curve 533/580)
RIDA®GENE Parasitic Stool Panel

**E. histolytica**

<table>
<thead>
<tr>
<th>RIDA®GENE</th>
<th>BNI PCR</th>
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<tbody>
<tr>
<td>Sensitivity:</td>
<td>100.0 %</td>
</tr>
<tr>
<td>Specificity:</td>
<td>100.0 %</td>
</tr>
<tr>
<td>PPV:</td>
<td>100.0 %</td>
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<tr>
<td>NPV:</td>
<td>100.0 %</td>
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**G. lamblia**

<table>
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<tr>
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<tr>
<td>PPV:</td>
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<tr>
<td>NPV:</td>
<td>98.9 %</td>
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RIDA®GENE Parasitic Stool Panel – Rotor Gene Q

E. histolytica (Orange)

Giardia lamblia (Green)
# RIDA®GENE Parasitic Stool Panel

## C. parvum

<table>
<thead>
<tr>
<th>RIDA®GENE</th>
<th>BNI PCR</th>
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<tbody>
<tr>
<td>Sensitivity:</td>
<td>96.4 %</td>
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<tr>
<td>Specificity:</td>
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<tr>
<td>PPV:</td>
<td>96.4 %</td>
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<tr>
<td>NPV:</td>
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## D. fragilis

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<tr>
<td>Sensitivity:</td>
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<td>Specificity:</td>
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<td>PPV:</td>
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<td>NPV:</td>
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RIDA®GENE Parasitic Stool Panel - Rotor Gene Q

C. parvum (Red)

D. fragilis (Melting curve Yellow)
RIDA®GENE Product Overview

**Gastro-intestinal Infections**
- Norovirus
- Norovirus I & II
- Rotavirus/Adenovirus Duplex
- Virus Stool Panel*
- Parasitic Stool Panel
- Bacterial Stool Panel
- EHEC/EPEC
- STEC
- EAEC
- ETEC/EIEC

**Hospital acquired Infections**
- MRSA
- PVL
- Hospital Stool Panel
- Clostridium difficile*
- CD Toxin A/B
- Clostridium difficile HyperTox

**Respiratory Infections**
- Influenza

*Available soon
# RIDA®GENE Product Line

- **Real-time (RT)-PCR** (Taqman probes)
- **High Sensitivity**
- **High Specificity**
- **Complete:** Contains all necessary components
- **Flexible:** Can be run on commonly used qPCR instruments
- **Efficient:** One PCR profile for DNA or RNA assays
- **Reliable:** Internal control
- **Validated:** CE & QCMD Quality assessment participation
Thank you
for your attention!