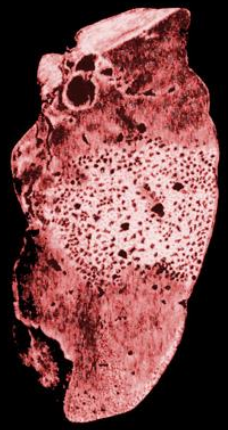
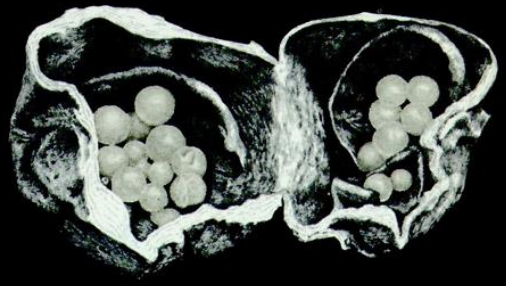
Echinococcosis
New Strategies for Serological and Molecular Diagnosis





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Casoni Skin Test

An intradermal test, used to for diagnosis.

Tomaso Casoni
(1880–1933)

https://web.stanford.edu/class/humbio103/ParaSites2003/Echinococcus/Diagnostics%20&%20Treatment2.htm
Man's arm showing positive skin test for hydatid disease. *Courtesy of CDC*

Diagnosis of Echinococcosis

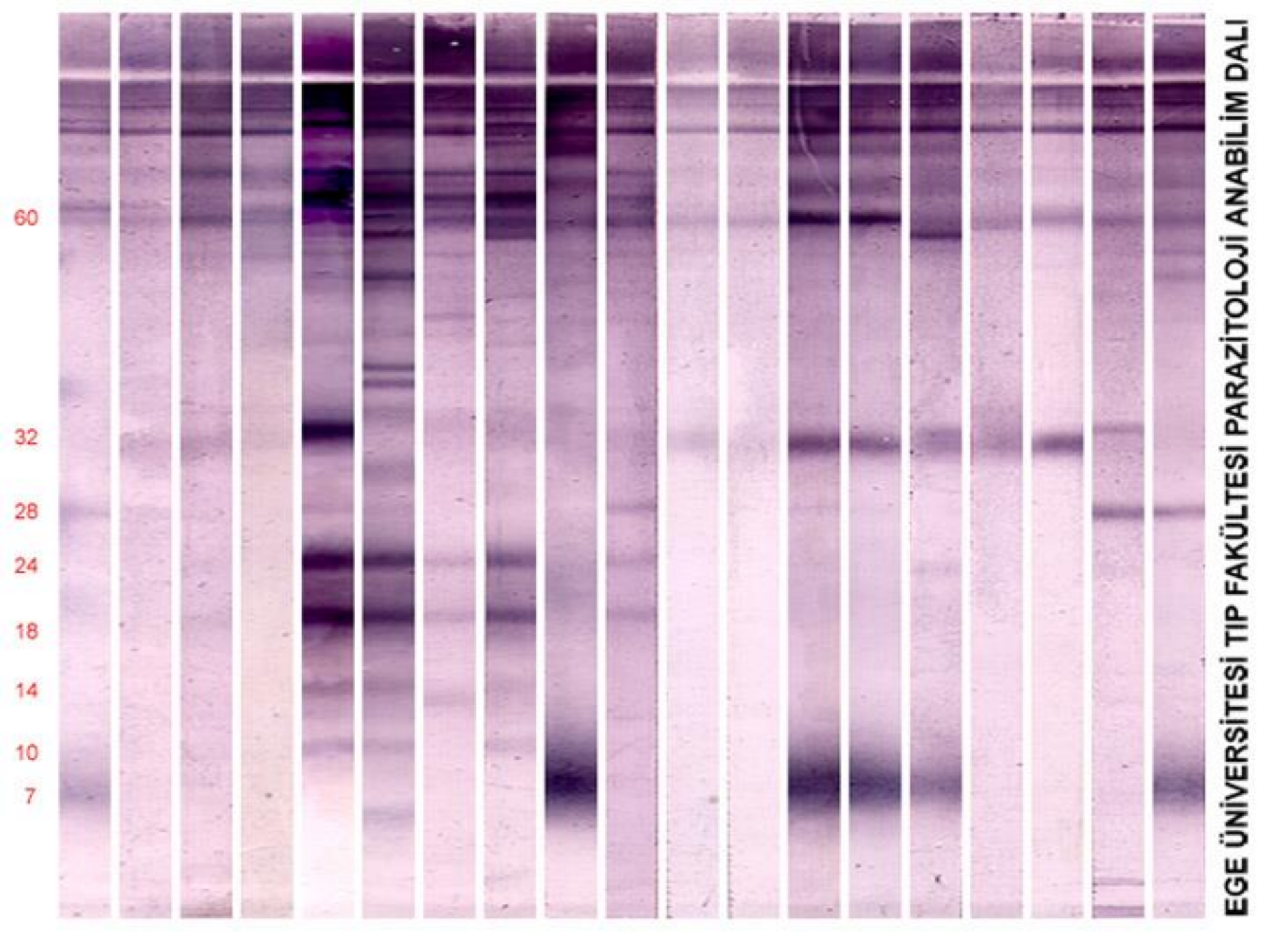
Primarily based on imaging techniques

- Imaging techniques
  - Ultrasonography
  - Radiology
  - CT scans and MRIs
- Serology
  - Antibody detection
  - Antigen detection
- Molecular
  - Mainly on the characterization of isolates
- Cyst aspiration or biopsy

Assays

Antibody Detection

- A specific antibody response, mainly of the IgG class
- Detectable IgA, IgM and IgE antibodies
- Almost all serological tests have been used
- Indirect hemagglutination test and enzyme-linked immunosorbent assay are the most widely used methods for detection of anti-Echinococcus antibodies.
- Using combinations of IHA and ELISA (or IFA) tests is recommended for serologic diagnosis
- A positive reaction is confirmed by immunoblot assay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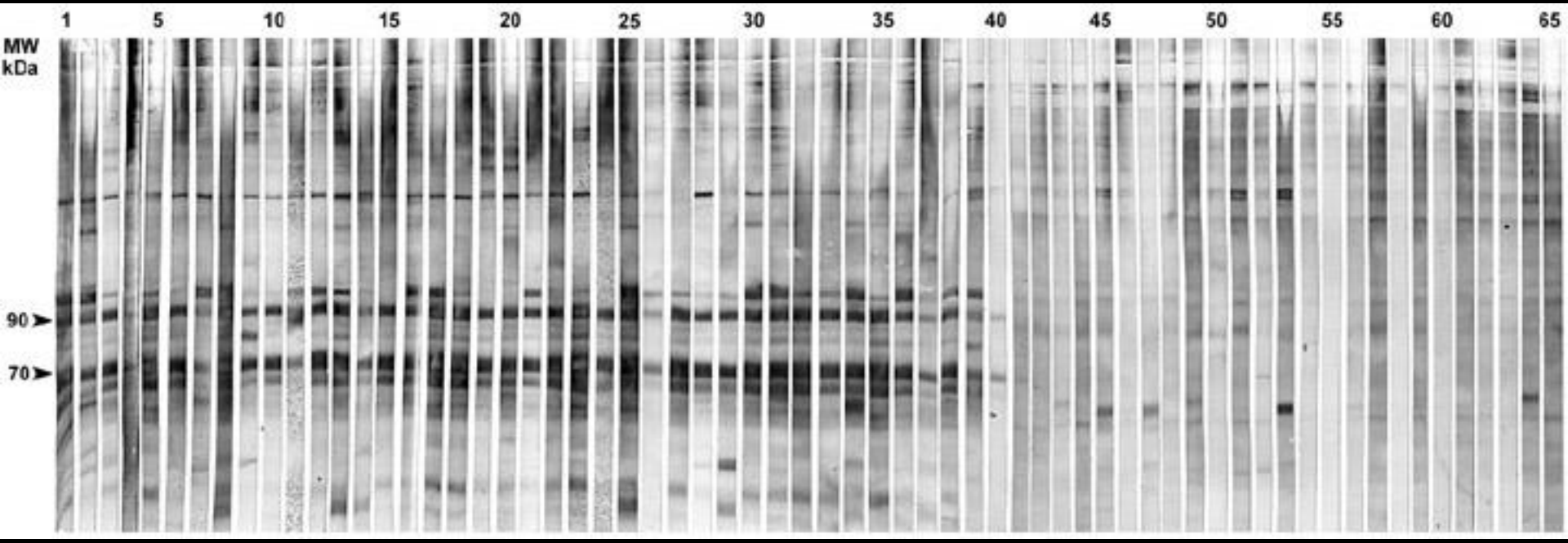
EGE ÜNİVERSİTESİ TIP FAKÜLTESİ PARAZİTOLOJİ ANABİLİM DALI

Alveolar Echinococcosis

Em70 and Em90



Parasite Antigens

Nature, purity and quality

- Protoscolex
- Hydatid cyst fluid
  - Antigen B, a polymeric lipoprotein
  - Antigen 5
- Recombinant antigens
  - Lack of a standardised test system due to native antigens
  - Recombinant antigens may be an alternative source for standardization.

Assays

Antigen Detection

- Generally applicable for laboratory research purposes only
- Monitoring disease?
- There are no diagnostic tests in routine practise

New Perspectives

Simple to perform and not time consuming

- New and better tests for screening
- Practical, where conventional serology techniques are unavailable
- Standardization of antigen
- Simple, precise and reproducible diagnostic tests were required

Recombinant antigens

The 8 kDa subunit of AgB is the most studied antigen

- Antigen B8/1 and B8/2
  - Provide the highest diagnostic sensitivity and specificity.
- *Echinococcus* protoscolex calcium binding protein (rEPC1)
- Multi-Epitope Antigens (MEA-8, MEA-20, MEA-26, MEA-36, MEA-49, and MEA-52)*
- rEmAgB3 suggested as a promising biomarker for serological assessment of AE patients. It is highly correlated with worm viability**
- Single defined molecule may not properly diagnose echinococcosis
- No significant difference between recombinant and native antigens

*Jiang et al, 2013. Serodiagnosis of the recombinant multi-epitope antigens from antigen B subunits of Echinococcus granulosus. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 31(6):438-42.

**Chun-Seob Ahna et al. 2015. An Echinococcus multilocularis Antigen B3 Proteoform That Shows Specific Antibody Responses to Active-Stage Alveolar Echinococcosis. J. Clin. Microbiol. 53(10): 3310-3317

Rapid Diagnostic Tests

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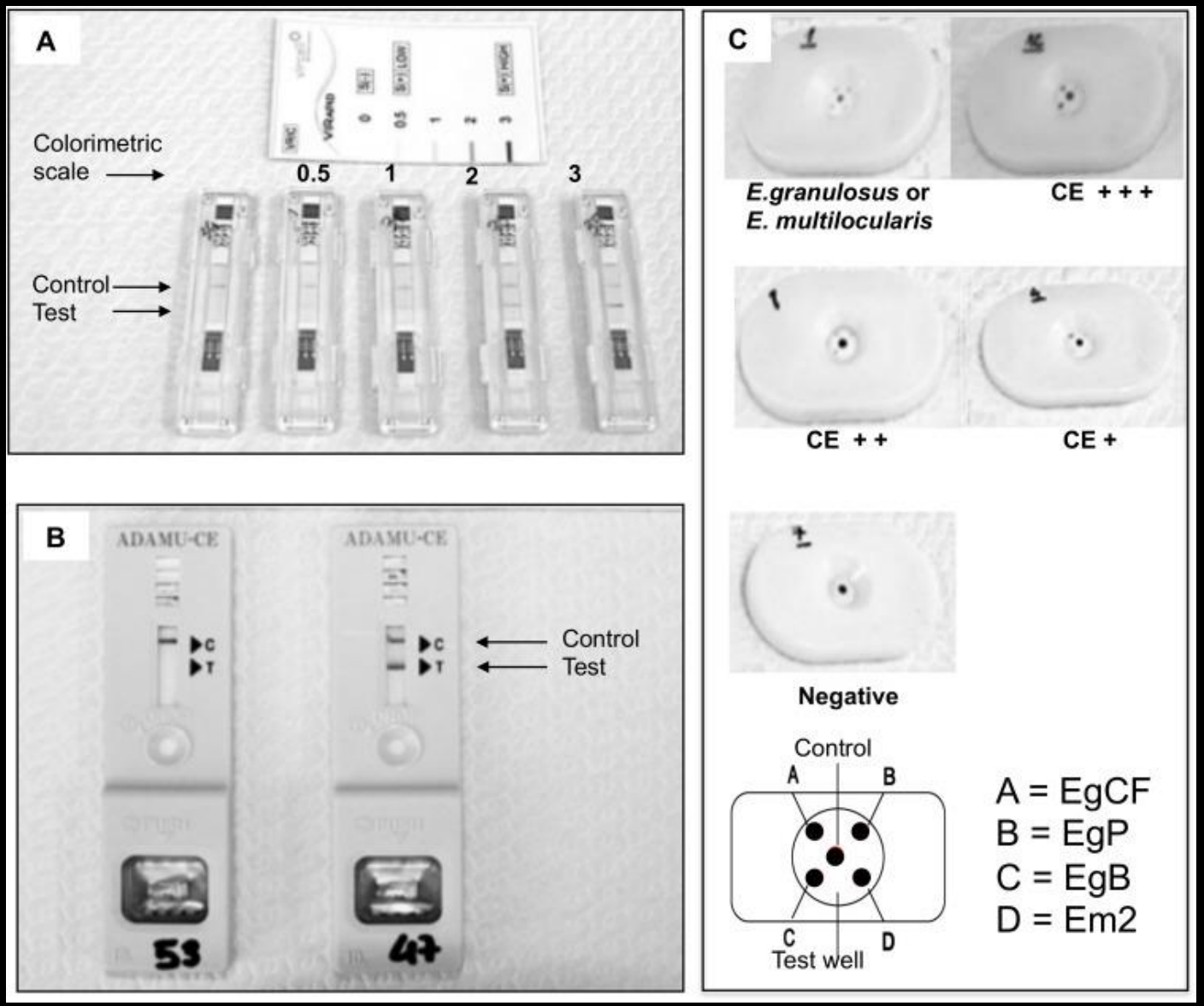
- VIRapid HYDATIDOSIS (Vircell, Salamanca, Spain)
  - Based on purified antigen B and antigen 5
- *Echinococcus* Dot Immunogold Filtration Assay (DIGFA, Unibiotest, Wuhan, China)
  - Based on purified cyst fluid, protoscolex antigen, antigen B and antigen Em2 of *E. multilocularis*
- ADAMU-CE (ICST, Saitama, Japan),
  - Based on recombinant antigen B
- rEm18-ICT for alveolar echinococcosis.



(A) VIRapid HYDATIDOSIS test and its semiquantitative colorimetric scale. **(B)** ADAMU-CE test. **(C)** DIGFA test and its diagnostic and semiquantitative colorimetric interpretation; EgCF = *E*. *granulosus* Cyst Fluid antigen, EgP = *E*. *granulosus* Protoscolex antigen, EgB = *E*. *granulosus* antigen B, Em2 = *E*. *multilocularis* antigen 2.

Tamarozzi et al. Comparison of the Diagnostic Accuracy of Three Rapid Tests for the Serodiagnosis of Hepatic Cystic Echinococcosis in Humans. PLoS Negl Trop Dis. 2016 Feb 12;10(2):e0004444.

Particle Gel Immunoaffinity Assay (PaGIA)

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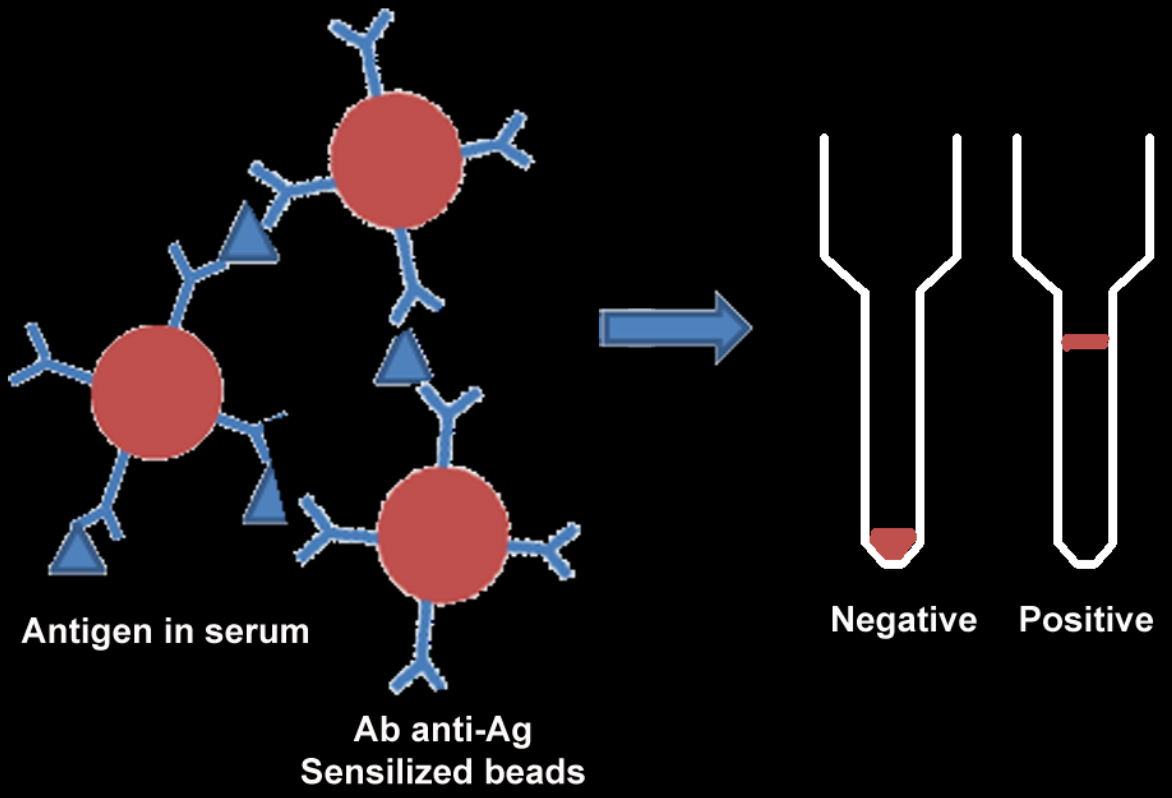
- PaGia is available to blood banks that use a gel centrifugation technology system.
- High-density polystyrene beads suspended in a gel similar to those used in transfusion medicine and is read like a blood group test.



Echinococcus granulosus PaGIA test

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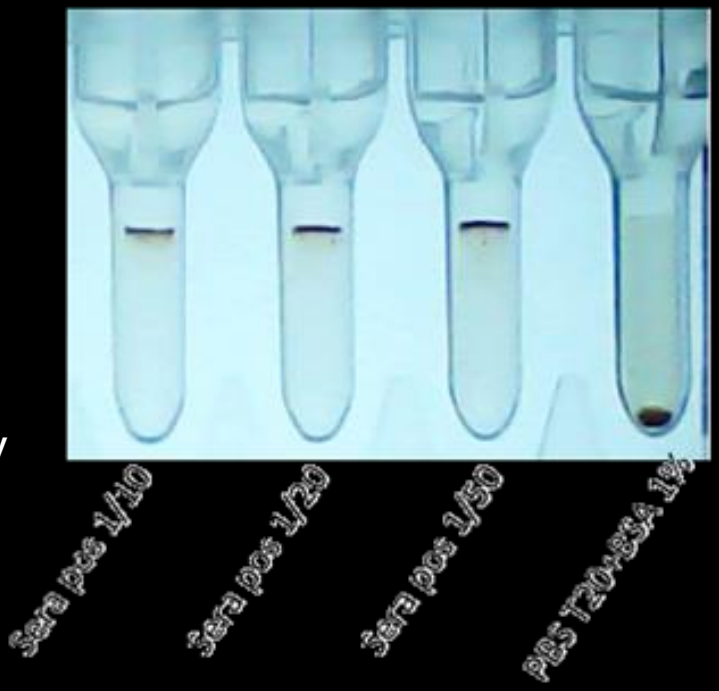
- A commercial *E. granulosus* PaGIA test (DiaPro) which based on recombinant antigens is underdevelopment.
- His tagged recombinant antigens: EgAg5, EgAgB8/2 and EgAgB8/1.
- Incubate 5 min, centrifuge 10 min
- Preliminary results showed acceptable sensitivity and specificity for detecting anti-*Echinococcus* antibodies.



Screening for Echinococcosis?

Why, whom, when to screen?

- Rationale: When detected and treated early the disease can be cured

- Ultrasound
- Serology

- US should be selected as the primary test in field studies*

*Ozkol et al. 2005. A discrepancy between cystic echinococcosis confirmed by ultrasound and seropositivity in Turkish children. Acta Trop. 93(2):213-6.

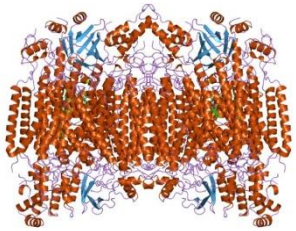
Molecular approaches

Species identfication, better understanding of pathogenesis

- Identification/discrimination of Echinococcus species in definitive and intermediate hosts.
- Better understanding of pathogenesis and disease
- Formalin-fixed paraffin-embedded liver tissue samples
- Multiplex PCR, which simultaneously using multiple specific primers in a single tube and detecting more than one target species, is an effective method for the identification of parasites.

Molecular approaches

Mitochondrial regions amplified and sequenced



- NADH dehydrogenase subunit 1 (nad1), 219 bp
- NADH dehydrogenase subunit 5 (nad5), 584 bp
- Cytochrome c oxidase subunit 1 (cox1), 471 bp
- Cytochrome c oxidase subunit 2 (cox2)
- 12S rRNA and Nad5 gene
- The 12S PCR was most sensitive of all tested*.
- A single PCR on the 12S gene proved to be very suitable for detection and specification of Taenia sp. and Echinococcus sp.

*Roelfsema JH, Nozari N, Pinelli E, Kortbeek LM. Novel PCRs for differential diagnosis of cestodes. Exp Parasitol. 2016 Feb;161:20-6.