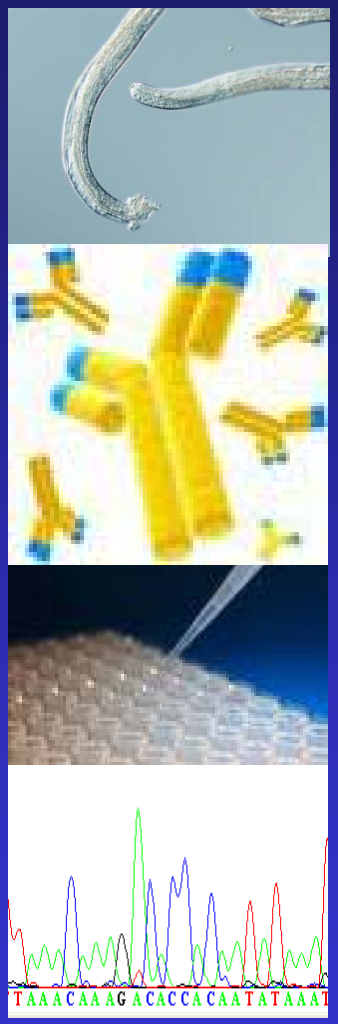

Current status of laboratory diagnosis of *Angiostrongylus cantonensis* infections

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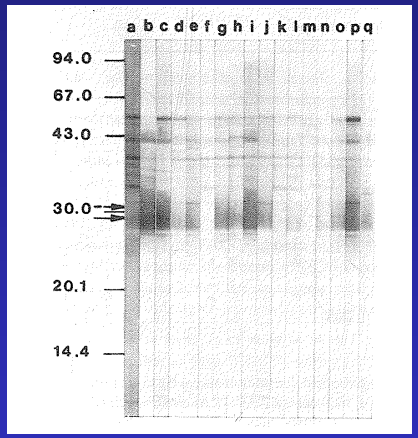
Parasitological diagnosis

- Direct observation of *A.cantonensis* in the CSF is rare
  - Third stage larvae may be present in CSF as early as 12 hours post infection
  - Juvenile worms may arrive in brain 1-3 weeks post infection
- May see worms in brain biopsy sections
- Rare infections of the eye have been reported

Juvenile worms (4th stage males) in CSF of a 9 mo child., Hawaii. (Courtesy of DPDx).

Serological diagnosis—historical perspective

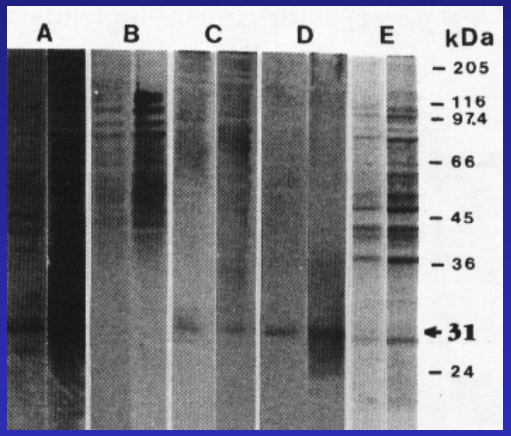
- 1992 Akao et al: Detection of antibodies to 29 and 31 kDa proteins in the extracts of female worms was reported



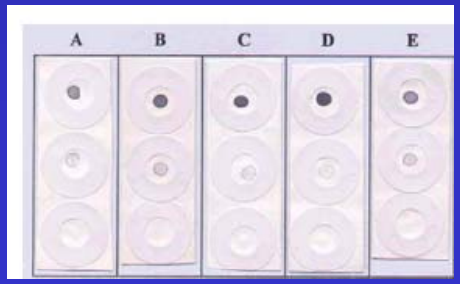
- 1996 Nuamtanong et al evaluated ELISA and blot:
  - ELISA 100% sensitive and 67% specific
  - Blot, 29kDa: 88% sens 47% spec
  - Blot, 31kDA: 68% sens, 82% spec

Serological diagnosis—historical perspective

- 2001, Eamsobhana et al purified the 31kDA proteins and used it in ELISA:
  - — 100% sens and 100% specific



- 2003, Eamsobhana et al used generated a dot blot assay using the purified 31kDA protein for use in regional hospital labs



Other targets for aby detection

- 2000 Chye et al: detected antibodies using an ELISA based on a MAb-purified 204 kDa antigen:
  - Serum:91% sensitive and 98% specific
  - CSF: 83% sensitive, 100% specific
  - Avg days post infection for all specimens tested = 15.3 days

CDC experience with 31kDA based serological methods

- Jamaica outbreak (2005):
  - Specimens collected 5-18 days post symptom onset were negative
  - Specimens from the same patients collected 31-45 days post onset were positive

- Recent evaluation of immunoblot using crude extract from female worms (2011):
  - 29/31 kDa blot : neither sensitive nor specific—cross reactions seen with sera from patients with toxocariasis, trichinellosis, echinococcosis

Antigen detection

- 1997, Chye et al : MAbs detect 204 kDa young adult antigen
  - Sensitivity = 81% (36/42) serum; 98% (41/42) CSF
  - Specificity = 100% serum and CSF (0/40)

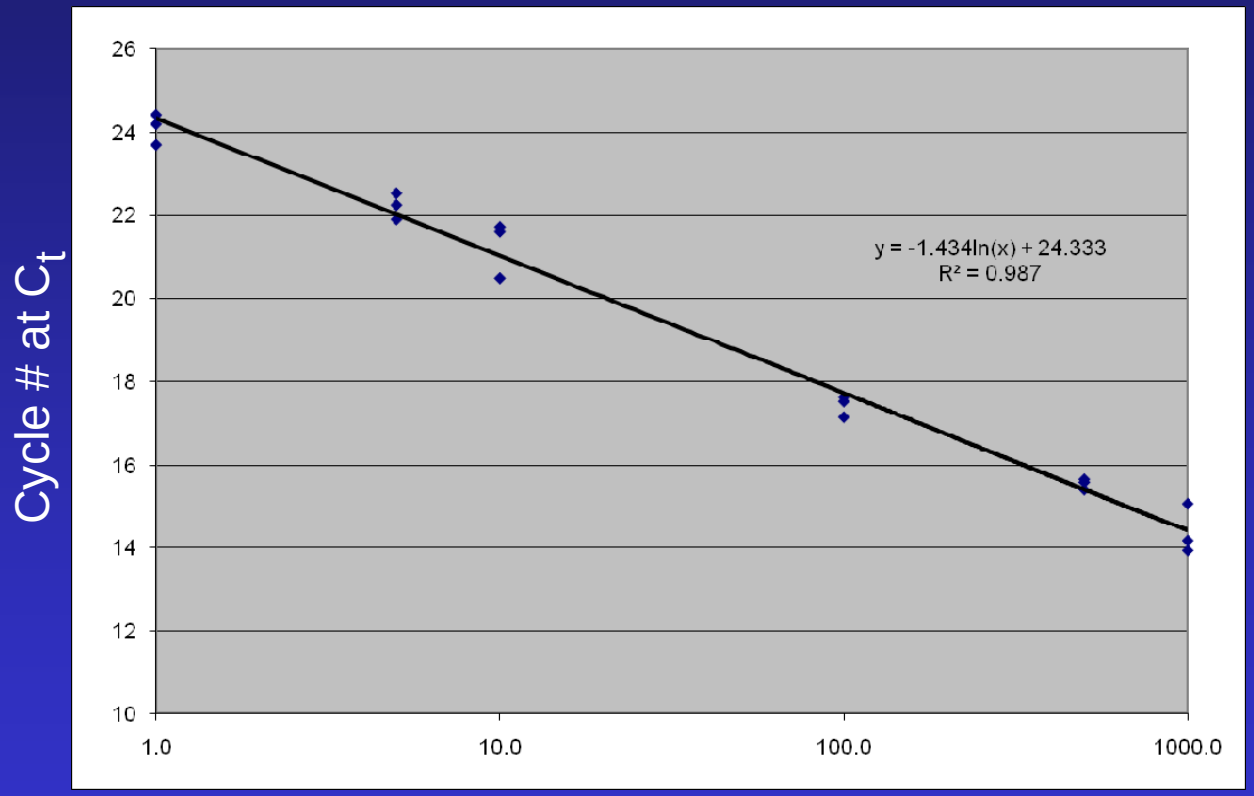
- 1997, Eamsobhana et al : MAb detects adult worm antigen in serum:
  - Sensitivity = 50% (5/10)
  - Specificity = 100% (0/103)

Molecular Diagnosis

- Sensitive and specific PCRs exist which can be used to detect parasite specific DNA in molluscan hosts tissues and human clinical specimens

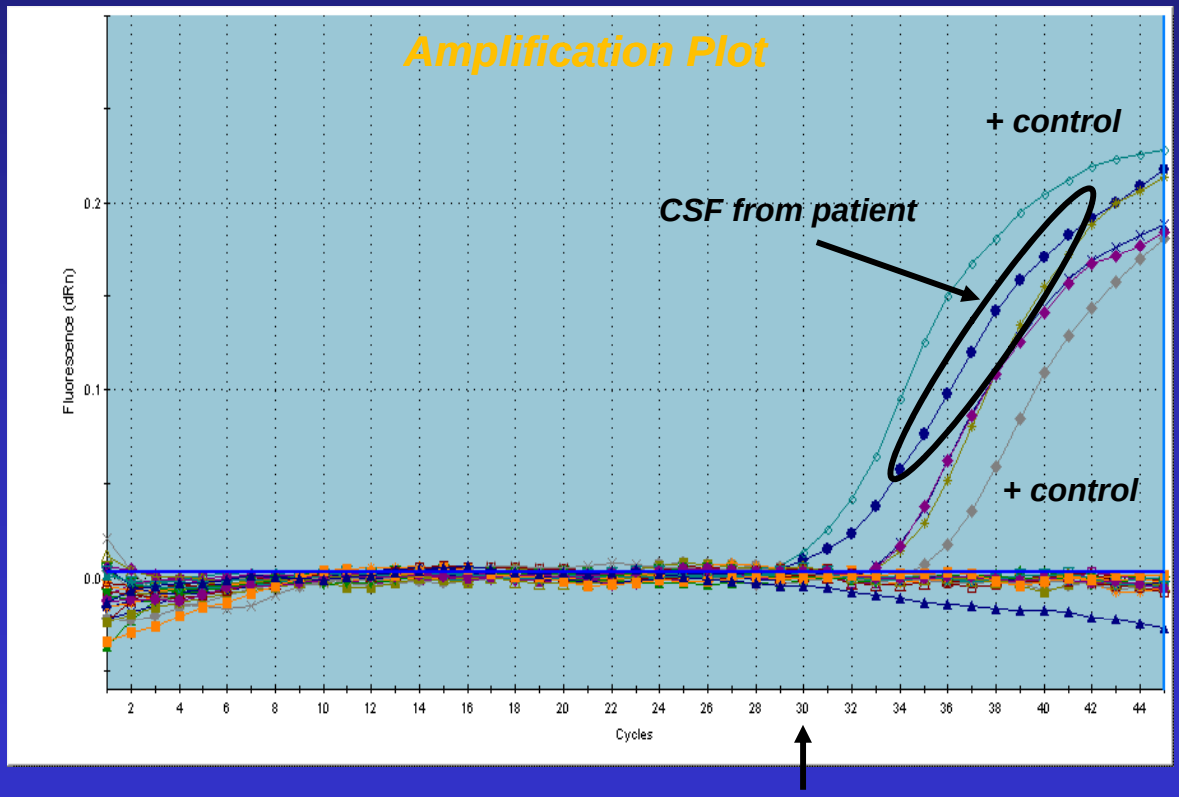
- CDC methods
  - 18S rRNA gene PCR/DNA sequencing
  - Species specific internal transcribed spacer (ITS-1) based TaqMan assay

ITS-1 real time PCR: Quantification



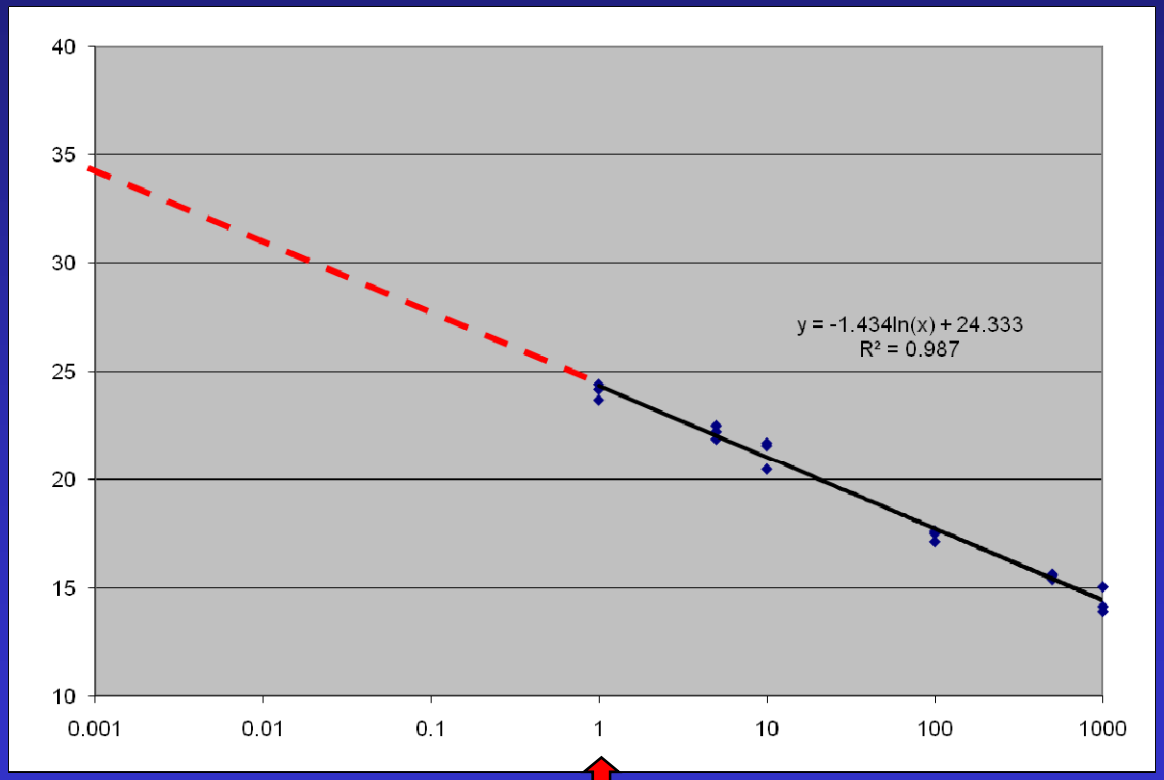
C_t for 1 larva is ~24 cycles

ITS-1 TaqMan PCR results using CSF

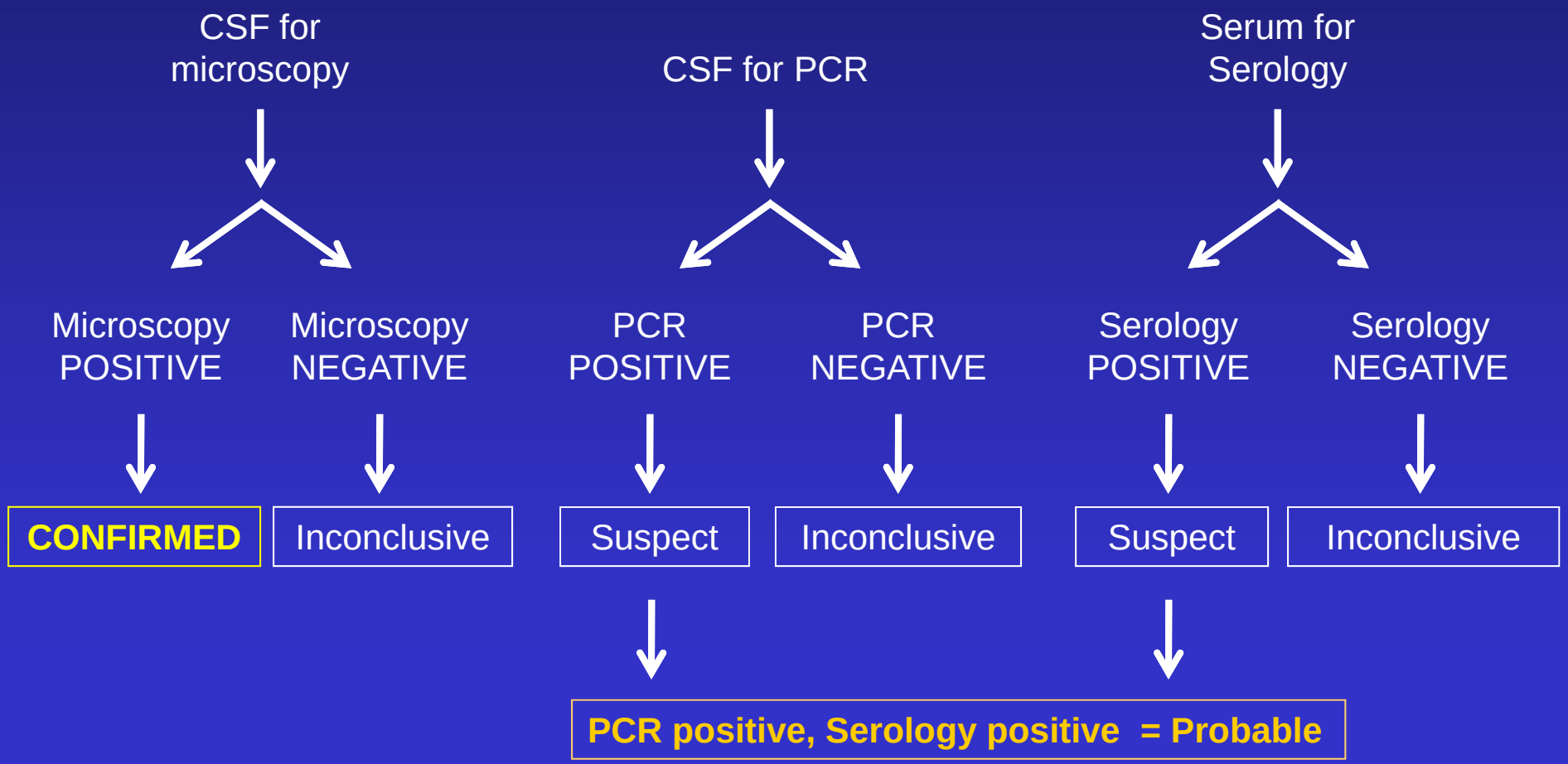


C_t of CSF from patient (~30) is higher than the C_t for 1 larva (~24)

ITS-1 TaqMan PCR detects <1 larva



Proposed Diagnostic Algorithm
in patients with eosinophilic meningitis



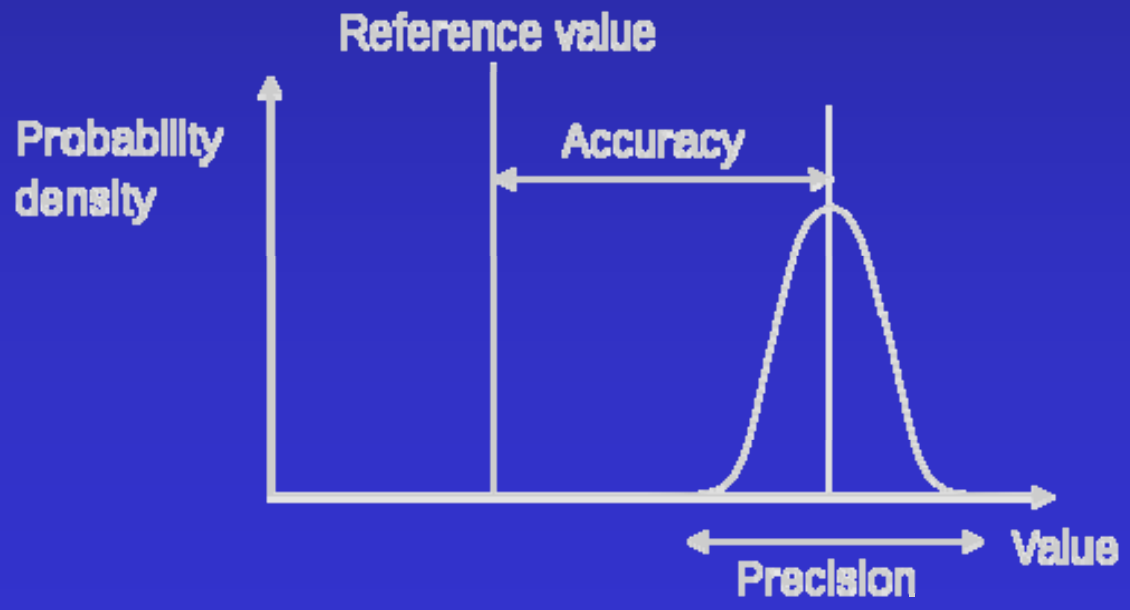
Lab Developed Tests (LDT)

- Testing may be performed for others, but not marketed to others (i.e., sold as a kit)
- Do not require FDA approval as do commercially developed and marketed tests
- Must go through verification procedures to establish performance characteristics before results can be used for decisions regarding patient care

Performance Characteristics

—Accuracy: How close is the test value to the "true" value (how close to the bull's eye)

—Precision: the reproducibility of a test result (how tight is the group)



Performance Characteristics

- Test sensitivity—the ability of a test to detect a substance especially at relatively low levels
- Test specificity—the test's ability to correctly detect or measure only the substance of interest and exclude other substances
- Both analytical and clinical performance must be determined

Federal Regulation of LDT

- CLIA Regulation enforced by CMS
- Applies to all facilities that perform
  - *"examination of materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings…."*
- CLIA is applicable to any result that may find its way into a patient chart
- Disclaimers do not absolve labs from this responsibility

Diagnostic dilemmas

- Direct observation of larvae is rare
- PCR if positive is informative; negative PCR results cannot be interpreted
- Serology may be negative in early infections
- Serology is valuable but is dependent on the quality of the test
- Antigen detection should be assessed to determine utility for clinical diagnosis
- Regulatory implications can impact test availability and use

References

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Questions?