



Evaluation of blood based assays for bovine TB in elk

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Parks Canada

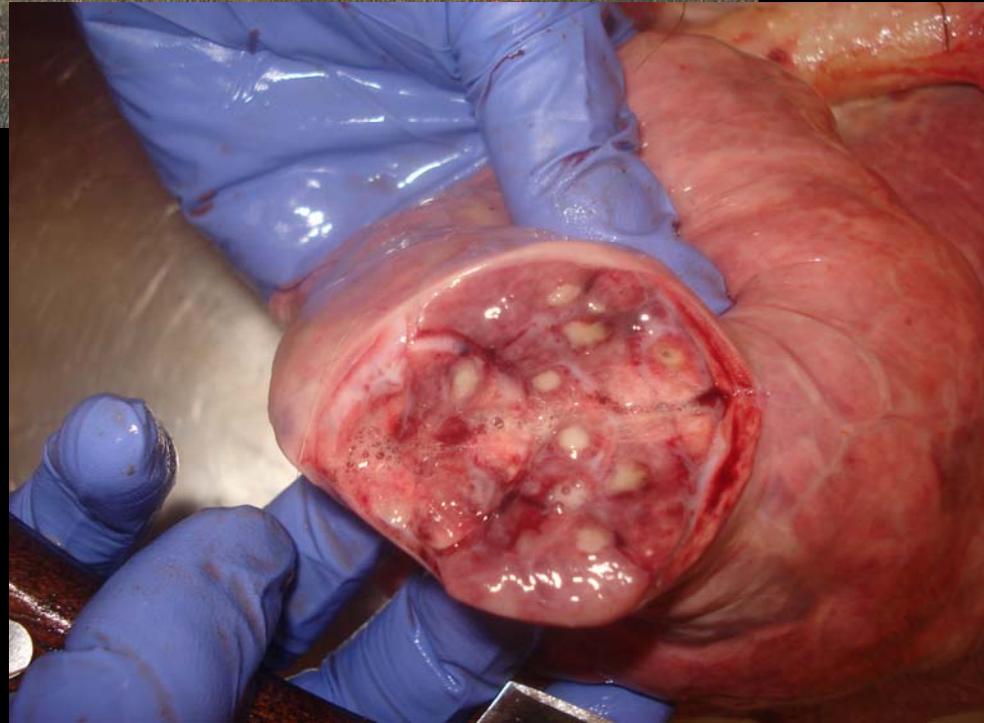
Saskatoon, Saskatchewan



Capture Protocol 2002-2007

- Helicopter net gun
- December to late April annually
- Short term VHF radio-collar with breakaways
- 50-70 cc of whole blood drawn, serum spun & frozen
- Positive elk & WTD recaptured 3 to 8 weeks later
- All suspect or + animals removed, necropsy and culture of multiple pooled lymph nodes (head, thorax, abdomen, body)
- Positive elk/WTD = + PCR on tissue or + culture for *M. bovis*





Testing protocol



- Lymphocyte Stimulation Test (LST) 2002-present
- Fluorescent Polarization Assay (FPA) 2002-present
- Lateral Flow Immunoassay (Rapid Test) 2005-present
- Multi-antigen Print Immunoassay (MAPIA) 2005-present
- Parallel interpretation (+ on any one test = + test)
- Test negative animals released (collar drop off) & assumed to be TB negative

Test Interpretation



Rapid test (lateral flow immunoassay)

- antibody based test on whole blood or serum 1+ to 3+

MAPIA (multi-antigen print immunoassay)

- antibody based test on serum only, pos. Or neg. Or suspect

12 recombinant or purified *M. Bovis* antigens

ESAT6,CFP10,MPB64,MPB59,MPB70,MPB83,16kda,38kda,

E6/P10,16/83,B-PPD, MBCF



Test Interpretation

Fluorescent polarization assay (FPA) – single Ag assay using MPB 70 Ag

- delta mp (compared to control)
- Positive (>15 mp) or Suspect (>10 mp)

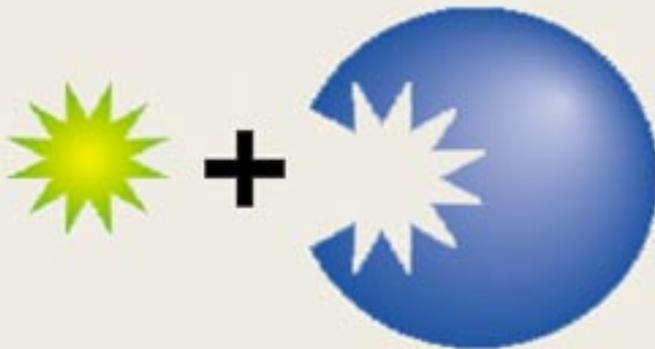
Lymphocyte Stimulation Test (LST) – Cell-mediated immunity

- delta CPM value of PPD, PPL, Johnin & *M. avium* PPD (compared to control: conA)
- Pos. = delta CPM $\geq 5,000$ and delta CPM of *M. avium* and Johnin is <66% of *M. bovis* delta CPM

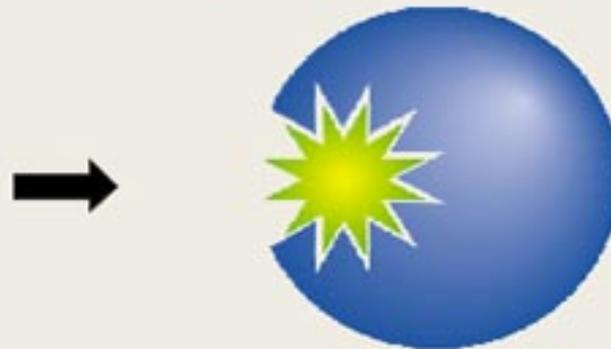
$$P \propto \text{Rotational Relaxation Time} = \frac{\eta V}{RT}$$

Where V is the molar volume of the rotating fluorophore, R is the gas constant, T the absolute temperature, and η the viscosity of the solution.

Low Polarization



High Polarization



How many were tested?

- 640 elk tested from 2002-2007, 40-164 annually
- 265 elk and 18 WTD available for necropsy
 - 226 elk & 15 WTD blood test positive
 - 27 elk & 3 WTD acute capture mortalities (cervical fractures)
- ~10% of samples unfit for testing (primarily LST).

Test evaluation criteria

Cutoff values chosen to maximize Se at expense of Sp

	Se	95% CI	N	Sp	95% CI	N	PPV	95% CI	NPV	95% CI
LST	91.3	73.2 - 97.6	23	77.7	71.4 - 82.9	197	17.7	13.8 - 22.4	99.4	98.1 - 99.8
FPA	43.5	25.6 - 63.2	23	65.2	58.4 - 71.4	201	6.2	3.83 - 9.78	95.6	93.8 - 97.0
RT	52.2	33.0 - 70.8	23	88	82.5 - 91.9	183	18.6	11.6 - 28.4	97.2	95.8 - 98.2
MAPIA	56.5	36.8 - 74.4	23	90.3	84.8 - 93.9	175	23.5	14.6 - 35.5	97.5	96.1 - 98.4

Comparative diagnostic values

	Weighted kappa*	Youden's Index	95% CI	Diagnostic odds ratio	95% CI
LST	0.84	0.69	0.56 to 0.82	36.5	8.2 to 161.8
FPA	0.12	0.09	-0.13 to 0.30	1.4	0.6 to 3.4
RT	0.43	0.40	0.19 to 0.61	8	3.1 to 20.3
MAPIA	0.50	0.47	0.26 to 0.68	12.1	4.6 to 32.0

* False negatives 100X more desirable than false positives

Specificity evaluation on “true” negative populations

Elk Island National Park (Alberta) 2004

94 semi free-ranging elk skin test negative

All negative on LST and FPA (100% specificity)

Rapid Test specificity tested on banked sera from elk
captured at the Ya Ha Tinda ranch in 2003

1 positive out of 40 sampled

Specificity: 97.5% (39/40)

Specificity evaluation on blood test negative elk

50 test negative (FPA, LST, PCR) elk submitted for necropsy, culture, histopath. In December 2003
MAPIA & RT completed retrospectively
Two positive animals identified on culture:

FPA – 96% (48/50)

LST – 98% (48/49) * one unfit sample

RT – 100% (50/50)

MAPIA – 98% (49/50)

Parallel interpretation (+ on either test)

	Se	Sp	Post-test Prob. +
RT & FPA	70	57.1	7.9
LST & FPA	95	47.4	8.7
MAPIA & FPA	70	59.6	8.4
LST & RT	95	67.3	13.3
LST & MAPIA	90	67.9	12.9
RT & MAPIA	65	81.4	47.5

Confounding factors

Different geographic areas tested (prob. of + test > in endemic area of RMNP)

- o Seasonal differences (Dec. to April)

- o Cutoff values chosen to maximize sensitivity at expense of specificity (to find all Btb positive animals)

- o Cross reaction with environmental mycobacteria?

- o Animals at different stages of infection

- o Low sensitivity of gold standard (culture) causing poor estimate of specificity

Blood test conclusions

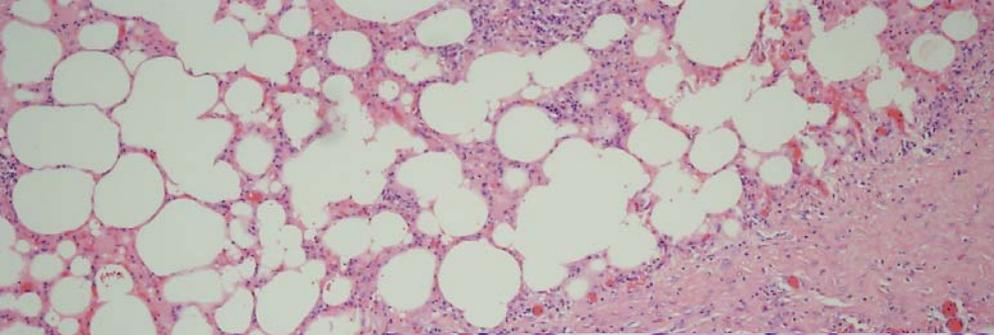
- 1) LST had best overall single test performance based on predictive values and Se/Sp. Difficulties with logistics getting samples to lab.
- 2) RT had highest Sp on evaluation of tests in a positive elk population, Se is low. Useful as a field test.
- 3) Problems with poor Se/Sp at low prevalence resulting in numerous false positives (based on culture)
- 4) Performance was less favourable than skin tests & other serological tests, but still allowed determination of geographic extent of outbreak
- 5) Combinations of tests will maximize Se and Sp (esp. CMI & Ab tests)

Gross Visible Lesions in lymph nodes & tissues of positive elk

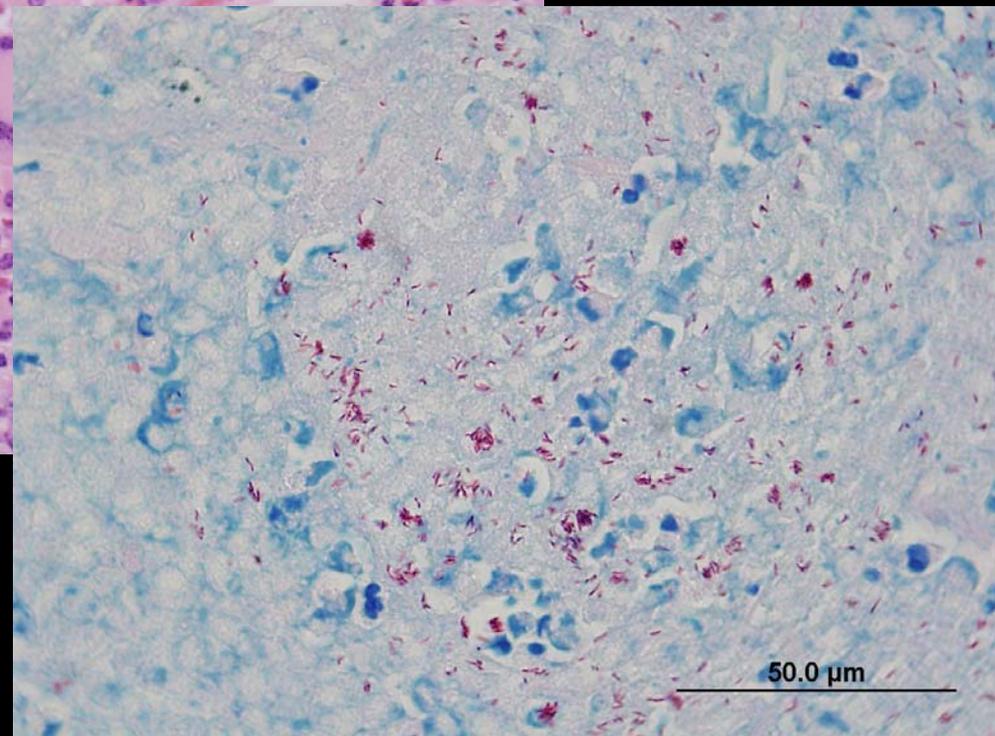
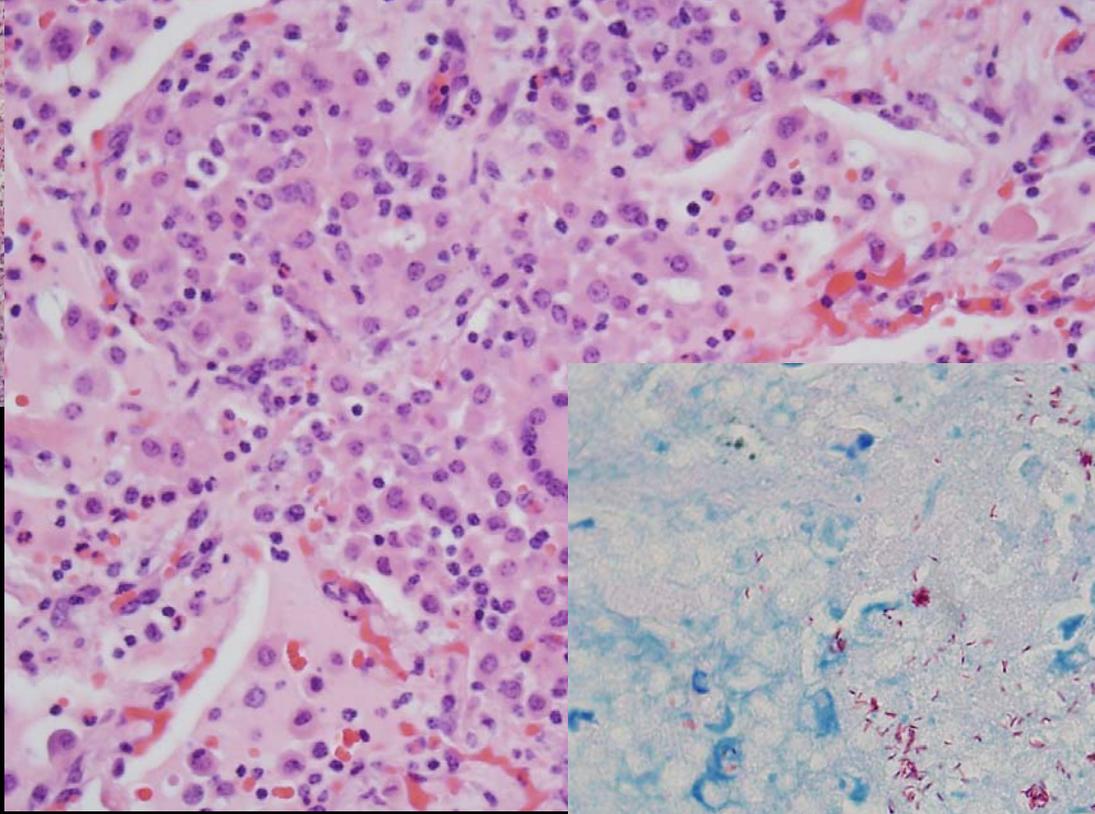
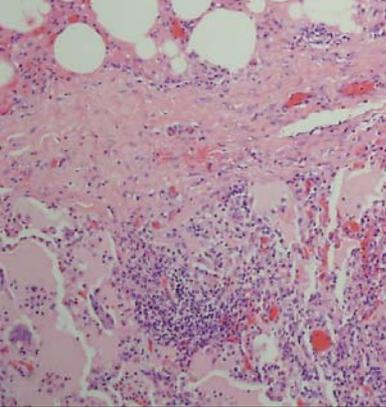
	Body		Head		Thorax		Abdomen	
Bulls	4/14	28.6%	9/14	64.3%	11/14	78.6%	3/14	21.4%
Cows	6/14	42.9%	5/14	35.7%	6/14	42.9%	1/14	7.1%
Total	10/28	35.7%	14/28	50.0%	17/28	60.7%	4/28	14.3%

1/28 (3.6%) culture -, PCR +





la



50.0 μ m

Apparent Prevalence Elk – blood tests

Elk – blood testing

23/650 = 3.5% overall 2002-2007 captures (based on culture + and PCR on lymph node)

All TB + elk on west side of park **23/375 = 6.1%**

Parallel interpretation of 3 tests: **47/230 = 20.4%**

Elk – hunter surveillance (1997-2007)

9/2900 = 0.3%

Apparent Prevalence WTD – blood tests

WTD – blood testing

$1/84 = 1.2\%$ overall 2005-2007 captures (based on culture + and PCR on lymph node)

All TB + WTD on west side $21/36 = 2.8\%$

Parallel interpretation of 3 tests: $16/84 = 19.0\%$

WTD – hunter surveillance (1997-2007)

$4/5000 = 0.1\%$

WTD Special Harvest March 2004

$2/225 = 0.9\%$



On the horizon....serum banking

Well-characterized, Btb-positive cervid sera very, very scarce!

Very expensive to experimentally infect cervids

Jeffrey Nelson, USDA Veterinary Services starting to build serum bank for cervid & cattle test development

Currently have ~1500 samples

Develop a common protocol for sample characterization

IDEXX and Enfer Group Ltd. (multiplex chemiluminescent ELISA) currently interested in utilizing this bank

PriTest SeraLyte Mbv™

MPB83 antigen with reflex supplemental testing

Utilized ferrite bead binding in sandwich format

93% Se, 100% Sp in UK badger study

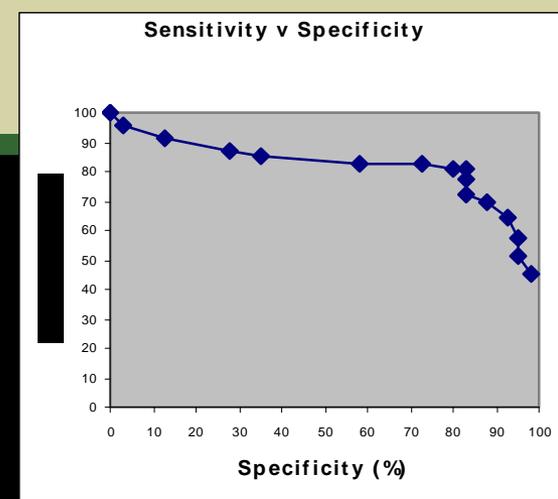
>90% Se, >95% Sp in cattle and humans

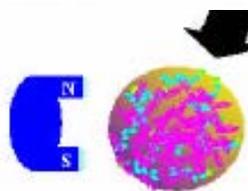
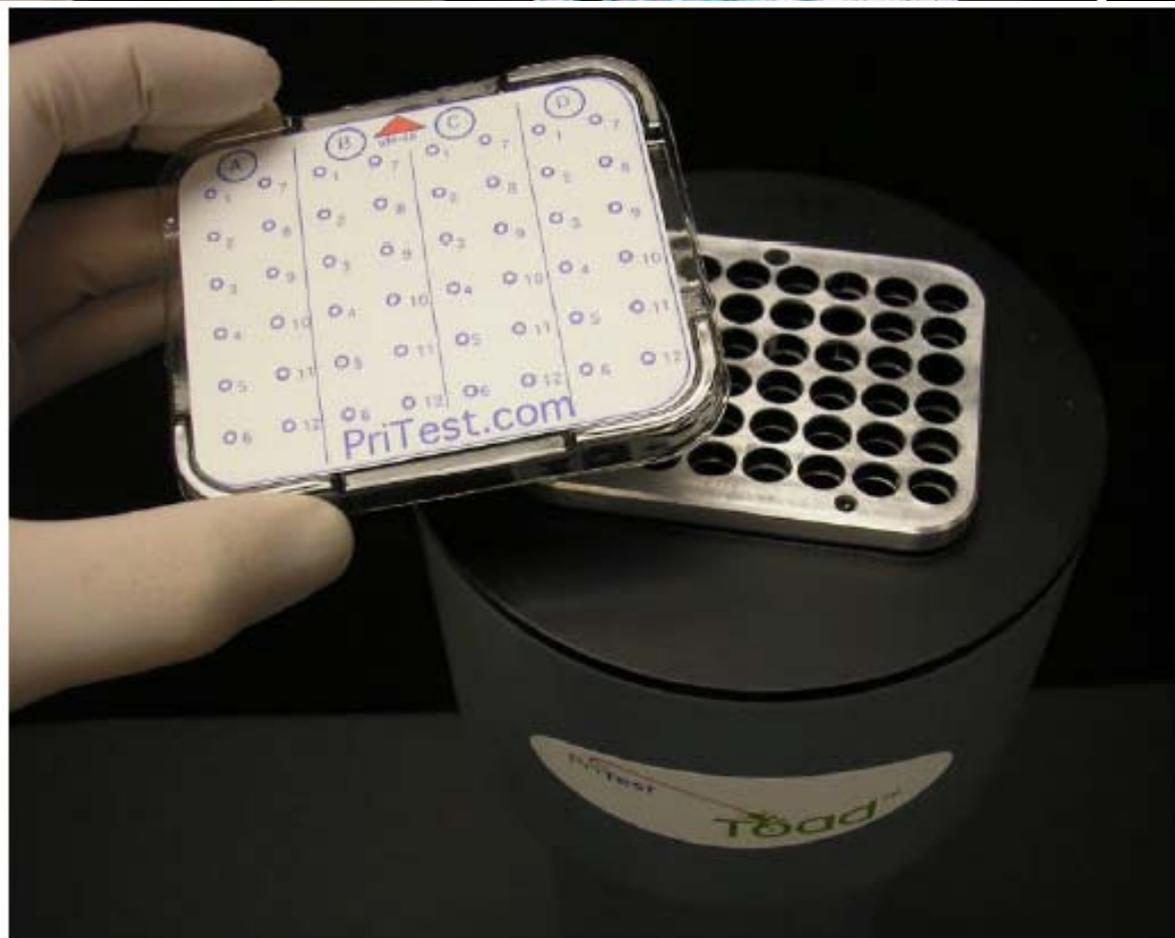
Completed in 2 hours or less

Portable platform

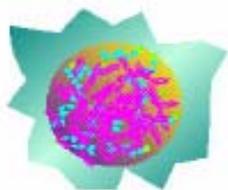
Optimization assay: 83% Se, 92% Sp

Blinded trial in August to confirm, continue to optimize

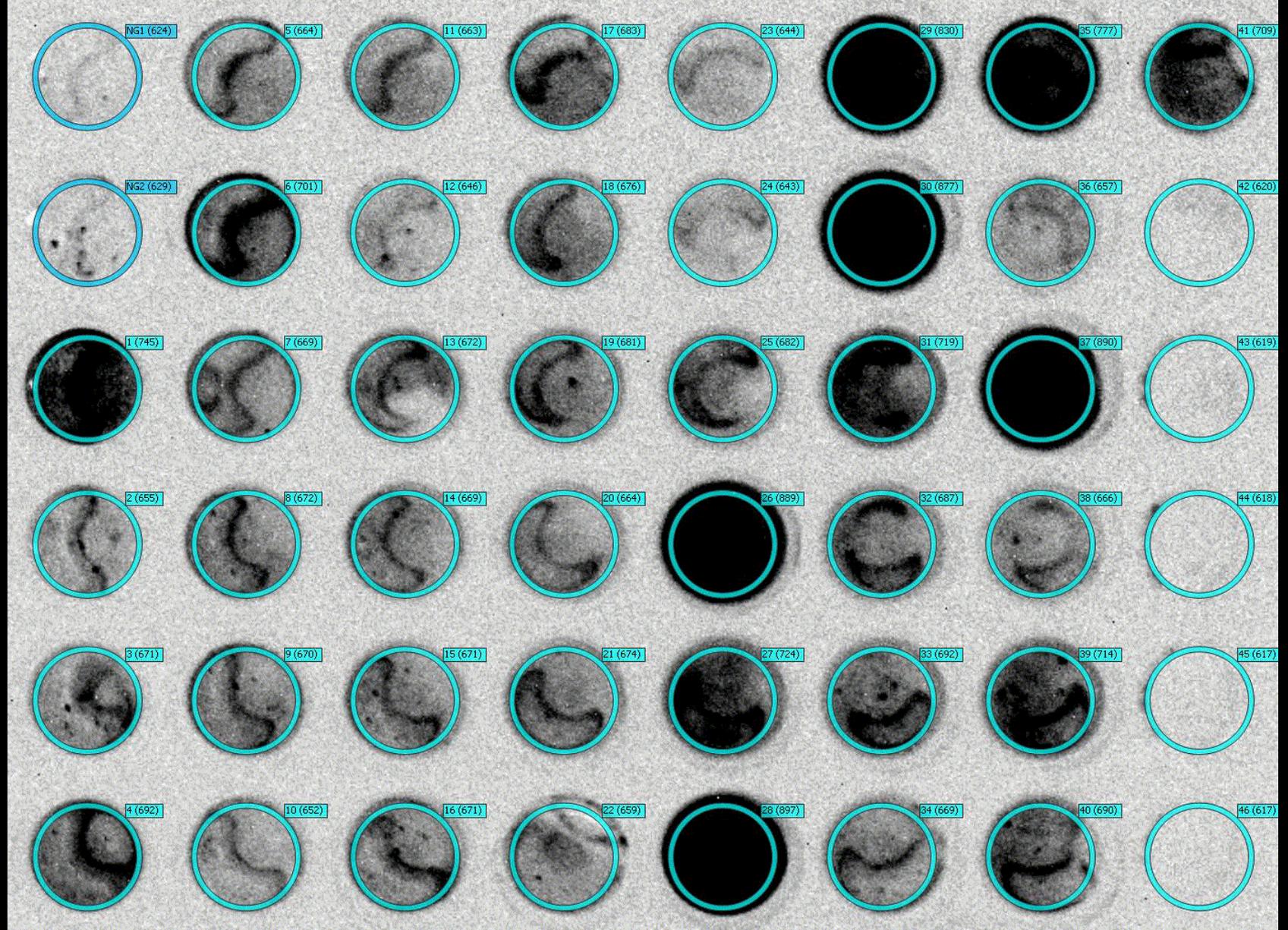




Particles are collected,
illuminated with reagent



Slide: cv_071908lg1 [Operator: Lawrence Green Date: 7/20/2008]





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